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(54) **Title:** NOVEL G PROTEIN-COUPLED RECEPTOR PROTEIN AND DNA THEREOF

(57) **Abstract:**

A human-origin G protein-coupled receptor protein, a peptide fragment or salts thereof; a nucleic acid encoding this receptor protein and its derivative; a nucleic acid having an antisense sequence to the base sequence encoding the above receptor protein and its derivative; a process for producing the above G protein-coupled receptor protein; a method for determining a ligand to the above G protein-coupled receptor protein; a method/kit for screening a compound capable of altering the binding of the ligand to the above G protein-coupled receptor protein; a compound obtained by the screening or its salt; and an antibody against the above G protein-coupled receptor protein, etc.

(57) Abstract [translated]

The present invention relates to a G protein-coupled receptor protein of human origin or salts thereof, peptide fragments of the receptor protein or salts of the peptide fragments, a nucleic acid encoding the receptor protein and derivatives of that nucleic acid, a nucleic acid having an antisense sequence to the base sequence encoding the receptor protein and derivatives of that nucleic acid, a process for producing the receptor protein, a method of determining a ligand for the receptor protein, a method/kit for screening for a compound capable of altering the binding of the ligand to the receptor protein; a compound obtained by the screening or salts of the compound, an antibody to the receptor protein, and the like.

The G protein-coupled receptor protein of human (human hippocampal) origin, or nucleic acids encoding the receptor protein, and derivatives thereof, of the present invention can be used (1) in determining ligands (agonists [tn 1]) for the receptor protein of the present invention; (2) as drugs for preventing and/or treating diseases related to insufficient function of the G protein-coupled receptor protein of the present invention; (3) as genetic diagnostic agents; (4) in quantifying ligands for the G protein-coupled receptor protein of the present invention; (5) in screening for compounds (agonists [tn 2], antagonists, etc.) that alter the binding of a ligand to the G protein-coupled receptor protein of the present invention; (6) as drugs for preventing and/or treating various diseases, those drugs containing a compound (agonist, antagonist) that alters the binding of a ligand to the G protein-coupled receptor protein of the present invention; (7) in quantifying the receptor protein of the present invention, peptide fragments thereof, or salts of the receptor protein or peptides thereof; (8) in neutralization by antibodies to the receptor protein of the present invention, to peptide fragments thereof, or to salts of the receptor protein or peptide fragments thereof; etc.

[The key for the PCT country abbreviations located here in the original was omitted from this translation.]

SPECIFICATION

Novel G protein-coupled receptor protein and DNA thereof

Technical Field

The present invention relates to a novel protein of human (human hippocampal) origin, salts thereof, DNA encoding the protein, and so on.

Background Art

Many hormones, neurotransmitters, and other physiologically active substances regulate the functions of the body via specific receptor proteins located in the cell membrane. Many of these receptor proteins mediate the transmission of intracellular signals via activation of a guanine nucleotide-binding protein (hereinafter sometimes referred to as "G protein") to which the receptor is coupled, and possess a common structure of 7 transmembrane domains. Therefore, such receptor proteins are generically referred to as "G protein-coupled receptor proteins" or "seven-transmembrane receptor proteins" (7-TMR proteins).

G protein-coupled receptor proteins exist on each functional cell surface of cells and organs of the body, and play important physiological roles as targets of molecules—such as hormones, neurotransmitters, and physiologically active substances—which regulate functions of those cells and organs. Receptors transduce signals into the cell by binding to physiologically active substances, and the signals elicit various reactions, which are activation or inhibition.

Determining the relationship between substances that regulate various complex functions in cells and organs of the body and their specific receptor proteins, in particular G protein-coupled receptor proteins, provides an extremely important means for elucidating various functions of cells and organs in the body and developing drugs closely related to those functions.

For example, regulation of physiological functions in various organs of the body takes place through regulation by many hormones, hormone-like substances, neurotransmitters, and physiologically active substances. In particular, physiologically active substances are present in a variety of sites in the body, and regulation of the physiological functions of the substances takes place through receptor proteins corresponding to each substance. There are still many unknown hormones, neurotransmitters, and other physiologically active substances in the body, and there is still much that has not yet been reported about the structure of their receptor proteins. Furthermore, for many known receptor proteins, whether or not subtypes exist is unknown.

Determining the relationship between substances that regulate complex functions in the body and their specific receptor proteins, in particular G protein-coupled receptor proteins, provides an extremely important means for developing drugs closely related to those functions. Furthermore, developing drugs by efficiently screening for agonists and antagonists of receptor proteins requires elucidation of the function of the receptor protein genes that are expressed in the body, and expression of those genes in suitable expression systems.

In recent years, research in which cDNA sequences are randomly analyzed is being pursued vigorously as a means of analyzing genes that are expressed in the body, and cDNA fragment sequences obtained this way are being entered in a database and made public as expressed sequence tags (ESTs). However, for many ESTs, there is only sequence information, and inferring their function is difficult.

Up to the present, as antagonists and agonists specific to G protein-coupled receptors, substances that inhibit the binding of physiologically active substances (namely, ligands) to the receptors and substances that by binding to the receptors cause signal transduction the same as that caused by physiologically active substances (namely, ligands) have been used as drugs that regulate functions in the body. Therefore, G protein-coupled receptor proteins are important in relation to their physiological expression in the body. But that is not all; newly discovering G protein-coupled receptor proteins that can become targets of drug development and cloning their genes (for example, cDNA) is an extremely important means of discovering specific ligands, agonists, and antagonists of novel G protein-coupled receptor proteins.

However, not all G protein-coupled receptors have been discovered. At present, there are many unknown G protein-coupled receptors and so-called "orphan receptors", which are G protein-coupled receptors whose ligands have not been identified, and there is a strong desire to search for new G protein-coupled receptors and elucidate their functions.

With their signal transducing effects as indexes, G protein-coupled receptors are useful in searching for new physiologically active substances (namely, ligands), and in searching for agonists and antagonists of the receptors. However, even if a ligand is not found, agonists or antagonists of a receptor can be produced by elucidating the physiological effects of the receptor through experiments in which the receptor is inactivated (knockout animals). Such ligands, agonists, antagonists, etc. of G protein-coupled receptors can be expected to be useful as agents for diagnosing, and as drugs for preventing/treating, diseases related to insufficient function of the receptors.

Furthermore, decreases or increases in the function of a G protein-coupled receptor in the body resulting from mutations in the gene for the receptor are often the cause of some disease. In such cases, not only can agonists or antagonists of the receptor be administered, but gene therapy can be used too, with that therapy consisting of introducing the receptor gene into the body (or a specific organ) or introducing receptor gene antisense nucleic acids. For gene therapy, the base sequence of the receptor [gene] is essential information for determining whether there are deletions or mutations in the gene, and the receptor gene can be used in agents for diagnosing, and drugs for preventing/treating, diseases related to insufficient function of the receptor.

The present invention provides a useful, novel G protein-coupled receptor protein as described above. That is, the invention provides a novel G protein-coupled receptor protein or peptide fragments thereof or salts thereof; polynucleotides (DNA, RNA, and their derivatives) including polynucleotides (DNA, RNA, and their derivatives) which encode the G protein-coupled receptor protein or a peptide fragment thereof; recombinant vectors containing the

polynucleotides; transformants which maintain the recombinant vectors; processes for producing the G protein-coupled receptor protein or salts thereof; antibodies to the G protein-coupled receptor protein, peptide fragments thereof, or salts thereof; compounds which alter the level of expression of the G protein-coupled receptor protein; methods of determining ligands for the G protein-coupled receptor; methods of screening for compounds (antagonists, agonists) or salts thereof which alter the binding of ligands to the G protein-coupled receptor protein; kits for the screening; compounds (antagonists, agonists) or salts thereof which alter the binding of ligands to the G protein-coupled receptor protein obtained by using the screening methods or screening kits; drugs formed containing compounds (antagonists, agonists) which alter the binding of ligands to the G protein-coupled receptor protein or compounds which alter the level of expression of the G protein-coupled receptor protein or salts thereof; etc.

Disclosure of the Invention

As a result of diligent research, the inventors, based on EST information from by the degenerated PCR method, succeeded in isolating cDNA encoding a novel receptor of human (human hippocampal) origin, and in analyzing the entire base sequence of the cDNA. After the base sequence was translated to an amino acid sequence, hydrophobicity plotting showed transmembrane domains 1 to 7, confirming that the proteins encoded by these cDNAs [tn 3] are 7-transmembrane-type G protein-coupled receptor proteins. Based on these findings, the inventors completed the present invention as a result of further research.

The invention relates to:

(1) A protein, or a salt thereof, which is characterized by containing an amino acid sequence the same as, or substantially the same as, the amino acid sequence of SEQ ID NO:1;

(2) A peptide fragment, or a salt thereof, of the protein of (1) above;

(3) A polynucleotide containing a polynucleotide which has a base sequence encoding the protein of (1) above;

(4) The polynucleotide of (3) above which is DNA;

(5) The polynucleotide of (3) above which has the base sequence of SEQ ID NO:2;

(6) A recombinant vector containing the polynucleotide of (3) above;

(7) A transformant which has been transformed by the recombinant vector of (6) above;

(8) A process for producing the protein of (1) above, or a salt thereof, characterized in that the protein of (1) above is caused to be produced by culturing the transformant of (7) above;

(9) An antibody to the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(10) The antibody of (9) above which is a neutralizing antibody that inactivates signal transduction of the protein of (1) above;

(11) A diagnostic agent which is formed containing the antibody of (9) above;

(12) A ligand for the protein of (1) above, or for a salt thereof, which can be obtained by using the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(13) A drug which is formed containing the ligand of (12) above;

(14) A method of determining a ligand for the protein of (1) above, or for a salt thereof, which is characterized by using the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(15) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein of (1) above or a salt thereof, [said method being] characterized by using the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(16) A kit for screening for a compound or a salt thereof which alters the binding of a ligand to the protein of (1) above or a salt thereof, [said kit being] characterized by containing the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(17) A compound or a salt thereof which alters the binding of a ligand to the protein of (1) above or a salt thereof, [said compound or salt thereof being] obtainable by using the screening method of (15) above or the screening kit of (16) above;

(18) A drug formed containing a compound or a salt thereof which alters the binding of a ligand to the protein of (1) above or a salt thereof, [said compound or salt thereof being] obtainable by using the screening method of (15) above or the screening kit of (16) above;

(19) A polynucleotide which hybridizes to the polynucleotide of (3) above under highly stringent conditions;

(20) A polynucleotide formed containing a base sequence, and a part of it, that are complementary to the polynucleotide of (3) above;

(21) A method of quantifying mRNA for the protein of (1) above characterized by using the polynucleotide of (3) above or a part of it;

(22) A method of quantifying the protein of (1) above characterized by using the antibody of (9) above;

(23) A method of diagnosing a disease related to a function of the protein of (1) above, [said method being] characterized by using the quantification method of (21) above or (22) above;

(24) A method of screening for a compound or a salt thereof which alters the level of expression of the protein of (1) above, [said method being] characterized by using the quantification method of (21) above;

(25) A method of screening for a compound or a salt thereof which alters the amount of the protein of (1) above in a cell membrane, [said method being] characterized by using the quantification method of (22) above;

(26) A compound or a salt thereof which alters the level of expression of the protein of (1) above, [said compound or salt being] obtainable by using the screening method of (24) above;

(27) A compound or a salt thereof which alters the amount of the protein of (1) above in a cell membrane, [said compound or salt being] obtainable by using the screening method of (25) above;

and so on.

Furthermore, the invention provides:

(28) The protein, or a salt thereof, of (1) above which is a protein containing ① the amino acid sequence of SEQ ID NO:1, the amino acid sequence of SEQ ID NO:1 having a deletion of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 9, and still more preferably several [1 to 5]), ② the amino acid sequence of SEQ ID NO:1 having an addition of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 10, and still more preferably several [1 to 5]), ③ the amino acid sequence of SEQ ID NO:1 having substitutions of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 10, and still more preferably several [1 to 5]) by other amino acids, or ④ an amino acid sequence that is a combination of the aforementioned amino acid sequences;

(29) The method of determining a ligand of (14) above which is characterized by the protein or salt thereof of (1) above, or the peptide fragment or salt thereof of (2) above, being caused to contact a test compound;

(30) The method of determining a ligand of (29) above wherein the ligand is, for example, angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin [*sic*], dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, α- and β-chemokines (for example, IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enterogastrin [*sic*], histamine, neurotensin, TRH, pancreatic polypeptide, or galanin;

(31) The screening method of (15) above which is characterized by comparing (i) the case in which the protein or salt thereof of (1) above or the peptide fragment or salt thereof of (2) above is brought into contact with a ligand, with (ii) the case in which the protein or salt thereof of (1) above or the peptide fragment or salt thereof of (2) above is brought into contact with a ligand and a test compound;

(32) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing the amount of binding of a labelled ligand to the protein or salt thereof of (1) above or to the peptide fragment or salt thereof of (2) above in (i) the case in which labelled ligand is brought into contact with the protein or salt thereof of (1) above or the peptide fragment or salt thereof of (2) above, and (ii) the case in which labelled ligand and a test compound are brought into contact with the protein or salt thereof of (1) above or the peptide fragment or salt thereof of (2) above;

(33) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing the amount of binding of a labelled ligand to a cell containing the protein of (1) above

in (i) the case in which labelled ligand is brought into contact with the cell, and (ii) the case in which labelled ligand and a test compound are brought into contact with the cell;

(34) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing the amount of binding of a labelled ligand to the membrane fraction of a cell containing the protein of (1) above in (i) the case in which labelled ligand is brought into contact with the membrane fraction of the cell, and (ii) the case in which labelled ligand and a test compound are brought into contact with the membrane fraction of the cell;

(35) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing the amount of binding of a labelled ligand to the protein expressed in the cell membrane of the transformant of (7) above by culturing the transformant in (i) the case in which labelled ligand is brought into contact with the protein, and (ii) the case in which labelled ligand and a test compound are brought into contact with the protein;

(36) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing protein-mediated cell stimulation activity in (i) the case in which a compound which activates the protein or salt thereof of (1) above is brought into contact with a cell containing the protein of (1) above, and (ii) the case in which a test compound and a compound which activates the protein or salt thereof of (1) above are brought into contact with a cell containing the protein of (1) above;

(37) A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein or salt thereof of (1) above which is characterized by measuring and comparing protein-mediated cell stimulation activity in (i) the case in which a compound which activates the protein or salt thereof of (1) above is brought into contact with the protein expressed in the cell membrane of the transformant of (7) above by culturing the transformant, and (ii) the case in which a test compound and a compound which activates the protein or salt thereof of (1) above are brought into contact with the protein expressed in the cell membrane of the transformant of (7) above by culturing the transformant;

(38) The screening method of (36) or (37) above wherein the compound which activates the protein of (1) above is angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin [*sic*], dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, α- and β-chemokines (for example, IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enterogastrin [*sic*], histamine, neurotensin, TRH, pancreatic polypeptide, or galanin;

(39) A compound, or a salt thereof, obtainable by a screening method of (31) to (38) above, which alters the binding of a ligand to the protein or salt thereof of (1) above;

(40) A drug characterized by containing a compound, or a salt thereof, obtainable by a screening method of (31) to (38) above, which alters the binding of a ligand to the protein or salt thereof of (1) above;

(41) The screening kit of (16) above which is characterized by containing a cell containing the protein of (1) above;

(42) The screening kit of (16) above which is characterized by containing the membrane fraction of a cell containing the protein of (1) above;

(43) The screening kit of (16) above which is characterized by containing protein expressed in the cell membrane of the transformant of (7) above by culturing the transformant;

(44) A compound, or a salt thereof, obtainable by using a screening kit of (41) to (43) above, which alters the binding of a ligand to the protein or salt thereof of (1) above;

(45) A drug characterized by containing a compound, or a salt thereof, obtainable by using a screening kit of (41) to (43) above, which alters the binding of a ligand to the protein or salt thereof of (1) above;

(46) A method for quantifying the protein of (1) above or the peptide fragment of (2) above or a salt thereof which is characterized by bringing into contact the antibody of (9) above, and the protein of (1) above or the peptide fragment of (2) above or a salt thereof;

(47) A method for quantifying the protein of (1) above or the peptide fragment of (2) above or a salt thereof in a liquid test sample, the method being characterized by competitively reacting the antibody of (9) above with the liquid test sample and labelled protein of (1) above or labelled peptide fragment of (2) above or labelled salt thereof, and measuring the percentage of labelled protein of (1) above or labelled peptide fragment of (2) above or labelled salt thereof that is bound to the antibody; and

(48) A method for quantifying the protein of (1) above or the peptide fragment of (2) above or a salt thereof in a liquid test sample, the method being characterized by reacting liquid test sample with antibody of (9) above insolubilized on a carrier and with labelled antibody of (9) above, either simultaneously or consecutively, and subsequently measuring the activity of the labeling agent on the insolubilized carrier;

and so on.

Brief Description of the Drawings

Figure 1 shows the DNA base sequence encoding the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention obtained in Example 1, and the amino acid sequence deduced from the base sequence (continued in Figure 2).

Figure 2 shows the DNA base sequence encoding the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention obtained in Example 1, and the amino acid sequence deduced from the base sequence (continuation of Figure 1).

Figure 3 shows a hydrophobicity plot of the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention prepared based on the amino acid sequence shown in Figure 1 and 2.

Best Mode for Carrying Out the Invention

The G protein-coupled receptor protein of the present invention (hereinafter sometimes abbreviated as "receptor protein") is a receptor protein containing an amino acid sequence which is the same, or substantially the same, as the amino acid sequence of SEQ ID NO:1 (the amino acid sequence of Figure 1 and 2).

The receptor protein of the present invention may be a synthetic protein or a protein originating in any cell of a human or [other] mammal (for example, guinea pig, rat, mouse, rabbit, pig, sheep, bovine, monkey, etc.) (for example, splenic cell, nerve cell, glia cell, pancreatic β cell, marrow cell, mesangial cell, Langerhans cell, epidermic cell, epithelial cell, endothelial cell, fibroblast, fibrocyte, myocyte, lipocyte, immunocyte [for example, macrophage, T cell, B cell, natural killer cell, mast cell, neutrophil, basophil, eosinophil, or monocyte], megakaryocyte, synovial cell, chondrocyte, osteocyte, osteoblast, osteoclast, mammary gland cell, hepatocyte, or interstitial cell; or a precursor cell, stem cell, or cancer cell of the aforementioned cells; etc.), [tn 4]or blood system cell; or originating in any tissue in which the cell is present, for example, brain, various brain sites (for example, olfactory bulb, amygdala, cerebral basal ganglia, hippocampus, thalamus, hypothalamus, subthalamic nucleus, cerebral cortex, medulla oblongata, cerebellum, occipital lobe, frontal lobe, temporal lobe, putamen, caudate nucleus, corpus callosum, substantia nigra), spinal cord, pituitary, stomach, pancreas, kidney, liver, gonad, thyroid gland, gallbladder, bone marrow, adrenal gland, skin, muscle, lung, digestive tract (for example, large intestine or small intestine), blood vessel, heart, thymus, spleen, submandibular gland, peripheral blood, peripheral blood cell, prostate, testicle, testis, ovary, placenta, uterus, bone, joint, skeletal muscle, and the like (especially brain and various brain sites).

Examples of amino acid sequences substantially the same as the amino acid sequence of SEQ ID NO:1 are amino acid sequences having homology to the sequence of SEQ ID NO:1 of at least about 50%, preferably at least about 70%, more preferably at least about 80%, still more preferably at least about 90%, most preferably at least about 95%, and so on.

Preferable proteins containing an amino acid sequence substantially the same as the amino acid sequence of SEQ ID NO:1 of the present invention are, for example, proteins having an amino acid sequence substantially the same as the amino acid sequence of SEQ ID NO:1, and having activity of substantially the same nature as that of the amino acid sequence of SEQ ID NO:1.

Examples of activity of substantially the same nature include ligand binding activity, signal information transducing activity, and the like. The phrase "substantially the same nature" indicates that the nature of the activities is the same. Therefore, although it is preferable that ligand binding activity, signal information transducing activity, and so on be equal (for example, about 0.01- to 100-fold, preferably about 0.5- to 20-fold, more preferably about 0.5- to 2-fold), the degree of these activities and quantitative factors such as the molecular weight of the proteins may differ.

Ligand binding activity, signal information transducing activity, and the like may be measured according to methods known per se; for example, measurement may be according to methods described hereinafter for determining and screening ligands.

As the receptor protein of the present invention, a protein containing ① the amino acid sequence of SEQ ID NO:1 having a deletion of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 10, and still more preferably several [1 to 5]), ② the amino acid sequence of SEQ ID NO:1 having an addition of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 10, and still more preferably several [1 to 5]), ③ the amino acid sequence of SEQ ID NO:1 having substitutions of 1, or 2 or more, amino acids (preferably about 1 to 30, more preferably about 1 to 10, and still more preferably several [1 to 5]) by other amino acids, or ④ an amino acid sequence that is a combination of the aforementioned amino acid sequences, etc. may be used.

The receptor protein of the present specification is represented according to the convention in peptide art; that is, the left end is the N-terminal (amino terminal) and the right end is the C-terminal (carboxyl terminal). In the receptor protein containing the amino acid sequence of SEQ ID NO:1, and other examples of the receptor protein of the present invention, the C-terminal is normally the carboxyl group (–COOH) or carboxylate ($–COO^-$), but it may be the amide ($–CONH_2$) or an ester (–COOR).

Examples of groups than can be used as R of the ester include a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C_{6-12} aryl group such as phenyl, α- naphthyl, etc.; a C_{7-14} aralkyl group such as a phenyl-C_{1-2} alkyl group (e.g., benzyl, phenethyl, etc.) or an α-naphthyl-C_{1-2} alkyl group (e.g. α-naphthylmethyl, etc.); and the like. In addition, a pivaloyloxymethyl group, which is a group widely used as [*sic*; in] esters for oral administration, can also be used.

When the receptor protein of the present invention has a carboxyl group (or carboxylate) at a position other than the C-terminal, the carboxyl group may be amidated or esterified, and such

amides or esters are within the scope of the receptor protein of the present invention. In this case, the above C-terminal esters, for example, may be used as the esters.

The receptor protein of the present invention also includes proteins described above in which the amino group of the N-terminal methionine residue is protected by a protective group (for example, a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g. a formyl group, acetyl, etc.), proteins described above in which the N-terminal side is cleaved in vivo and the glutamyl group that is formed is pyroglutaminated, proteins described above in which substituents on side chains of amino acids in the molecule (for example, –OH, –SH, amino groups, imidazole groups, indole groups, guanido groups, etc.) are protected with appropriate protective groups (for example, a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g. a formyl group, acetyl, etc.), conjugated proteins such as so-called glycoproteins, to which sugar chains are bound, and so on.

Examples of embodiments of the receptor protein of the present invention are receptor proteins of human origin (more preferably of human hippocampal origin) containing the amino acid sequence of SEQ ID NO:1.

As a peptide fragment of the receptor protein of the present invention (hereinafter sometimes abbreviated to "peptide fragment"), any fragment may used provided that it is a peptide fragment of the receptor protein of the present invention; for example, among receptor protein molecules of the present invention, a part of the receptor protein molecule of the present invention which is exposed to the outside of a cell membrane and which has receptor binding activity may be used.

Specifically, a peptide fragment of the receptor protein having the amino acid sequence of SEQ ID NO:1 is a peptide containing a part which has been analyzed to be an extracellular domain (hydrophilic domain) in the hydrophobicity plotting analysis shown in Figure 3. A peptide including a hydrophobic domain as a part can similarly be used. A peptide which contains each domain separately may be used, and a peptide which contains a plurality of domains simultaneously [*sic*] is also acceptable.

As the number amino acids in a peptide fragment of the present invention, a peptide having an amino acid sequence of at least 20, preferably at least 50, and more preferably at least 100 amino acids from among the aforementioned constituent amino acid sequences of the receptor protein of the present invention, and the like, is preferable.

The phrase "amino acid sequences substantially the same" indicates amino acid sequences having homology to these sequences of at least about 50%, preferably at least about 70%, more preferably at least about 80%, still more preferably at least about 90%, and most preferably at least about 95%.

Here, the phrase "activity of substantially the same nature" has the same meaning as above, and "activity of substantially the same nature" may be measured as above.

In the peptide fragment of the present invention, the above amino acid sequence may have a deletion of 1, or 2 or more, amino acids (preferably about 1 to 10, more preferably several [1 to 5]); or the amino acid sequence may have an addition of 1, or 2 or more, amino acids (preferably about 1 to 20, more preferably about 1 to 10, still more preferably several [1 to 5]); or the amino

acid sequence may have substitutions of 1, or 2 or more, amino acids (preferably about 1 to 10, more preferably several, still more preferably about 1 to 5) by other amino acids.

In the peptide fragment of the present invention, the C-terminal is normally the carboxyl group (–COOH) or carboxylate ($–COO^-$), but like the receptor protein of the present invention described above, the C-terminal may be the amide ($–CONH_2$) or an ester (–COOR). As R in the ester, the same things used above and the like may be used.

The same as the receptor protein of the present invention described above, peptide fragments of the present invention also include peptides in which the amino group of the N-terminal methionine residue is protected by a protective group, peptides in which the N-terminal side is cleaved in vivo and the Gln that is formed is pyroglutaminated, peptides in which substituents on side chains of amino acids in the molecule are protected with appropriate protective groups, conjugated proteins such as so-called glycoproteins, to which sugar chains are bound, and so on.

In the peptide fragment of the present invention, the C-terminal is normally the carboxyl group (–COOH) or carboxylate ($–COO^-$), but like the receptor protein of the present invention described above, the C-terminal may be the amide ($–CONH_2$) or an ester (–COOR). As R in the ester, the same things used above and the like may be used [*sic*; tn 5].

As salts of the receptor protein or peptide fragments thereof of the present invention, physiologically acceptable salts with acid or base can be mentioned as examples, and in particular, physiologically acceptable acid addition salts are preferred. Examples of such salts that can be used are salts with inorganic acids (for example, hydrochloric acid, phosphoric acid, hydrobromic acid, and sulfuric acid) and salts with organic acids (for example, acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, and benzenesulfonic acid).

The receptor protein or a salt thereof of the present invention may be produced from the aforementioned tissues or cells of humans or [other] mammals according to methods of purifying receptor proteins known per se, or may be produced by culturing a transformant transformed with DNA encoding the receptor protein, as described hereinafter. The receptor protein or a salt thereof may also be produced by, or in accordance with, the protein synthesis method described hereinafter.

When the protein or salt is produced from tissues or cells of humans or [other] mammals, the cells or tissues are homogenized and then extracted with, for example, an acid. The extract can then be purified and isolated by combining chromatographic techniques, such as reversed phase chromatography, ion exchange chromatography, and the like [*sic*; tn 6].

In the synthesis of the receptor protein of the present invention, a peptide fragment thereof, or their salts or amides, normal commercially available resins for protein synthesis can be used. Examples of these resins include chloromethyl resin, hydroxymethyl resin, benzhydrylamino resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamino resin, PAM resin, 4-hydroxymethyl-methylphenylacetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, and 4-(2',4'-dimethoxyphenyl-Fmoc-

aminoethyl)phenoxy resin. Using a resin like these, amino acids with suitably protected side-chain functional groups are serially condensed with the α-amino group, on the resin, in the order corresponding to the amino acid sequence of the objective protein by various condensation techniques which are known per se. At the final reaction, the protein is cut out from the resin, and at the same time, the various protective groups are removed. Then, the intramolecular disulfide-forming reactions are carried out in highly dilute solution, and the objective proteins or amides thereof are obtained.

In relation to the above condensation of protected amino acids, various activators useful for protein synthesis can be utilized, with carbodiimide reagents being especially preferred. The carbodiimide reagents that can be used include DCC, N,N'-diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminoprolyl)carbodiimide, and so on. For activation by these reagents, the protected amino acid and a racemization inhibitor (e.g. HOBt, HOOBt) can be directly added to the resin, or the protected amino acid can be added to the resin after activation beforehand as symmetric acid anhydride, or HOBt ester or HOOBt ester.

The solvent used for activation of protected amino acids and conjugation of them to the resin can be suitably selected from among the solvents known to be useful for protein condensation reactions. Examples of solvents that can be used include acid amides such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, etc.; halogenated hydrocarbons such as methylene chloride, chloroform, etc.; alcohols such as trifluoroethanol, etc.; sulfoxides such as dimethyl sulfoxide, etc.; ethers such as pyridine, dioxane, tetrahydrofuran, etc.; nitriles such as acetonitrile, propionitrile, etc.; esters such as methyl acetate, ethyl acetate, etc.; and suitable mixtures of these solvents. The reaction temperature can be suitably selected from the range known to be useful for protein-forming reactions, usually the range of about -20°C to about 50°C. The activated amino acid derivative is normally used in a 1.5- to 4-fold excess. When the results of a test using a ninhydrin reaction show the condensation to be insufficient, sufficient condensation can be achieved by repeating the condensation reaction without eliminating the protective groups. When sufficient condensation cannot be achieved by repeating the reaction, unreacted amino acid can be acetylated by using acetic anhydride or acetylimidazole.

Examples of protective groups for the amino group of the starting compound that can be used include Z, Boc, tert-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxy-benzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulfenyl, diphenylphosphinothioyl, Fmoc, and so on.

The carboxyl group can be protected, for example, by alkyl esterification (for example, straight-chain, branched, or cyclic alkyl esterification to methyl, ethyl, propyl, butyl, tert-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 2-adamantyl, or other alkyl ester), aralkyl esterification (for example, esterification to benzyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, benzhydryl, or other aralkyl ester), phenacyl esterification,

benzyloxycarbonylhydrazidation, tert-butoxycarbonylhydrazidation, tritylhydrazidation, and so on.

The hydroxyl group of serine can be protected by, for example, esterification or etherification. The groups suitable for esterification include carboxylic acid-derived groups such as a lower alkanoyl group like acetyl, etc., an aroyl group like benzoyl, etc., a benzyloxycarbonyl group, an ethoxycarbonyl group, and so on. The groups suitable for etherification include a benzyl group, a tetrahydropyranyl group, a t-butyl group, and so on.

Examples of the protective group for the phenolic hydroxyl group of tyrosine that can be used include Bzl, Cl_2-Bzl, 2-nitrobenzyl, Br-Z, tert-butyl, and so on.

Examples of the protective group for the imidazole group of histidine that can be used include Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc, and so on.

Examples of the starting compound with activated carboxy groups that can be used include the corresponding acid anhydride, azide, and active ester (e.g. esters with alcohols such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccinimide, N-hydroxyphthalimide, and HOBt), and so on. An example of the starting compound with activated amino group is the corresponding phosphoric acid amide.

Examples of methods for removal (elimination) of protective groups that can be used include catalytic reduction in a hydrogen stream in the presence of a catalyst such as Pd black or Pd on carbon; acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, or a mixture of them; treatment with a base such as diisopropylethylamine, triethylamine, piperidine, piperazine, or the like; and reduction with sodium in liquid ammonia. The above elimination reaction by treatment with acid is generally conducted at a temperature of about -20°C to 40°C. In the acid treatment, it is effective to add a cation acceptor such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethyl sulfide, 1,4-butanedithiol, 1,2-ethanedithiol, or the like. The 2,4-dinitrophenyl group used for protecting the imidazole group of histidine is removed by treatment with thiophenol, and the formyl group used for protecting the indole group of tryptophan is removed not only by the aforementioned acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol or the like, but also by alkali treatment with dilute sodium hydroxide solution, dilute ammonia, or the like.

The method of protecting any functional group that should not participate in the reaction of starting compounds, the protective group, the method of eliminating the protective group, the method of activating the functional group to be involved in the reaction, and so on can be suitably selected from known groups and methods.

An alternative method for obtaining the amide form of the protein includes, for example, protecting the α-carboxyl group of the C-terminal amino acid by amidation, extending the peptide (protein) chain to a desired length toward the N-terminus, preparing a protein consisting of this peptide chain with just the protective group of its N-terminal α-amino acid removed and a protein with just the protective group of the C-terminal carboxyl group removed, and

condensing these two proteins in a mixed solvent such as the one mentioned above. The details of the condensation reaction are the same as described above. After purification of the protected protein obtained by the condensation, the desired crude protein can be obtained by eliminating all the protective groups by the procedures described above. The desired protein amide is then obtained by purifying the crude protein by making free use of various known purification techniques, and lyophilizing the main fraction.

A method for obtaining the ester form of the protein includes, for example, preparing an amino acid ester by condensing the α-carboxyl group of the C-terminal amino acid with a desired alcohol, and obtaining the desired protein ester by the same procedure described for the protein amide.

A peptide fragment or a salt thereof of the present invention may be produced by per se known procedures for peptide synthesis or by cleaving the protein of the present invention with an appropriate peptidase. The process for synthesizing the peptide may be, for example, a solid-phase synthesis or a liquid-phase synthesis. That is, the objective peptide can be produced by condensing a peptide fragment or amino acid capable of constituting the protein of the present invention with the residual part, and when the product has a protective group, eliminating that group. Examples of known methods of condensation and protective group elimination include those described in (1) to (5) below.

(1) M. Bodanszky and M. A. Ondetti: *Peptide Synthesis*, Interscience Publishers, New York, 1966

(2) Schroeder and Luebke: *The Peptide*, Academic Press, New York, 1965

(3) Nobuo Izumiya et al.: *Pepuchido Gosei no Kiso to Jikken* [translation: Fundamentals of, and Experiments in, Peptide Synthesis], Maruzen, 1975

(4) Haruaki Yajima and Shumpei Sakakibara: *Seikagaku Jikken Koza 1, Tanpakushitu no Kagaku IV* [translation: Biochemical Experiments, Series 1, Protein Chemistry IV], 205, 1977

(5) Haruaki Yajima (ed.): *Zoku Iyakuhin no Kaihatsu*, Dai 14 kan, *Pepuchido Gosei* [translation: Development of Drugs, Continued; Volume 14, Peptide Synthesis], Hirokawa Shoten

After the reaction, the peptide fragment of the present invention can be purified and isolated by a combination of conventional purification techniques, such as solvent extraction, distillation, column chromatography, liquid chromatography, and recrystallization. When the peptide fragment obtained by the above methods is a free compound, it can be converted to an appropriate salt by known methods; alternatively, when the fragment is obtained as a salt, it can be converted to the free compound by known methods.

The polynucleotide encoding the receptor protein of the present invention may be any polynucleotide (DNA or RNA, preferably DNA) that contains a base sequence encoding the receptor protein of the present invention described above. The polynucleotide is DNA, or RNA such as mRNA etc., that encodes the receptor protein of the present invention, and the DNA or RNA may be single- or double-stranded. If double-stranded, the polynucleotide may be double-stranded DNA, double-stranded RNA, or a DNA:RNA hybrid. If single-stranded, the polynucleotide may be the sense strand (namely, the coding strand) or the antisense strand (namely, the noncoding strand).

Using a polynucleotide encoding the receptor protein of the present invention, mRNA of the receptor protein of the present invention can be quantified, for example, by the known method described in "New PCR and Its Application" [in Japanese], *Jikken Igaku Zokan* [Experimental medicine, special edition], 15(7), 1977, or by an analogous method.

DNA encoding the receptor protein of the present invention may be genomic DNA, a genomic DNA library, cDNA derived from the tissues or cells mentioned above, a cDNA library derived from the tissues or cells mentioned above, or synthetic DNA. The vector used for a library may be any of bacteriophage, plasmid, cosmid, phagemid, or the like. Furthermore, using a total RNA fraction or an mRNA fraction prepared from the tissues or cells mentioned above, direct amplification can be carried out by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR).

Specifically, the DNA encoding the receptor protein of the present invention may be, for example, any DNA containing the base sequence of SEQ ID NO:2, or any DNA having a base sequence that hybridizes to the base sequence of SEQ ID NO:2 under highly stringent conditions, and encoding a receptor protein having activity (e.g., ligand binding activity, signal information transducing activity, etc.) of substantially the same nature as that of the receptor protein of the present invention.

As DNA that can hybridize to the base sequence of SEQ ID NO:2, DNA that, for example, contains a base sequence that has homology to the base sequence of SEQ ID NO:2 of at least about 70%, preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% may be used.

Hybridization may be carried out by a per se known method or an analogous method, for example, the method described in *Molecular Cloning*, 2nd (J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989). When a commercially available library is used, the hybridization can be carried out by the method described in the accompanying instructions. And more preferably, hybridization can be carried out under highly stringent conditions.

The phrase "highly stringent conditions" indicates, for example, conditions of a sodium concentration of about 19 to 40 mM, preferably about 19 to 20 mM, and a temperature of about 50°C to 70°C, preferably about 60°C to 65°C. In particular, the most preferable conditions are those of a sodium concentration of about 19 mM and a temperature of about 65°C.

More specifically, DNA having the base sequence of SEQ ID NO:2, and the like, can be used as DNA encoding a receptor protein having the amino acid sequence of SEQ ID NO:1.

The phrase "a polynucleotide formed containing part of a DNA base sequence encoding the receptor protein of the present invention or part of a base sequence complementary to that DNA" is used with the meaning including not only DNA encoding a peptide fragment of the present invention described below, but also including RNA.

According to the present invention, antisense polynucleotides (nucleic acids) capable of inhibiting the replication or expression of G protein-coupled receptor protein genes may be designed and synthesized based on information on the base sequence of cloned or determined DNAs encoding G protein-coupled receptor proteins. Such antisense polynucleotides (nucleic acids) are capable of hybridizing with RNA of G protein-coupled receptor protein genes to inhibit the synthesis or function of the RNA, or of regulating and controlling expression of G protein-coupled receptor protein genes via interaction with RNA related to the receptor protein. Polynucleotides complementary to selected sequences of G protein-coupled receptor protein-related RNA, and polynucleotides specifically hybridizable with G protein-coupled receptor protein-related RNA, are useful in regulating and controlling expression of G protein-coupled receptor protein genes in vitro and in vivo, and in treating or diagnosing diseases and the like. The term "corresponding" means homologous to, or complementary to, a particular sequence of the polynucleotide, base sequence, or nucleic acid including the gene. Between a peptide (protein) and a polynucleotide, base sequence, or nucleic acid, the term "corresponding" normally refers to the amino acids of a peptide (protein) whose instructions were derived from the polynucleotide (nucleic acid) sequence or its complement. The G protein-coupled receptor protein gene 5'end hairpin loop, 5'end 6-base-pair repeats, 5'end untranslated region, polypeptide translation initiation codon, protein-coding region, ORF translation initiation codon, 3'untranslated region, 3'end palindrome region, and 3'end hairpin loop may be selected as preferable target regions, but any region in the G protein-coupled receptor gene may be a target.

As for the relationship between the objective nucleic acid and a polynucleotide complementary to at least a portion of the target region, the relationship with a polynucleotide capable of hybridizing with the target material, the thing known as "antisense" is possible [*sic*; tn 7]. Antisense polynucleotides may be polydeoxynucleotides containing 2-deoxy-D-ribose, polydeoxynucleotides containing D-ribose, other types of polynucleotide which are an N-glycoside of a purine or pyrimidine base, other polymers having nonnucleotide backbones (for example, commercially available protein nucleic acid or synthetic sequence-specific nucleic acid polymers), or other polymers containing special linkages (provided that the polymers contain a nucleotide having a configuration that allows base pairing or base attachment like those seen in DNA and RNA). They may be double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA, or a DNA:RNA hybrid; and they may be unmodified polynucleotides (or unmodified oligonucleotides), and even may be ones having added known modifications, for example known-in-the-art ones with labels, ones with caps attached,

methylated ones, ones with an analogue substituted for one or more of the naturally occurring nucleotides, ones with intramolecular nucleotide modifications, for example ones having uncharged linkages (e.g. methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), ones having charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), for example ones having side-chain groups such as proteins (nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.) or saccharides (e.g., monosaccharides, etc.) or the like, ones having intercalators (e.g., acridine, psoralen, etc.), ones containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), ones containing alkylators, and ones having modified linkages (e.g., α-anomeric nucleic acids, etc.) [tn 8]. The terms "nucleoside", "nucleotide", and "nucleic acid" include not only purine and pyrimidine bases, but also may include [compounds] having other heterocyclic bases that have been modified. Such modified entities may be methylated purines and pyrimidines, acylated purines and pyrimidines, or other heterocycles. Modified nucleotides and nucleotides [*sic*] may also be modified on the sugar moiety; for example, one or more of the hydroxyl groups may be replaced with halogens or aliphatic groups, or may be converted to functional groups such as ether, amine, and the like.

The antisense polynucleotides (nucleic acids) of the present invention are RNA, DNA, or modified nucleic acid (RNA, DNA). Examples of modified nucleic acids are sulfur derivatives and thiophosphate derivatives of nucleic acids, and modified nucleic acids resistant to poly- and oligonucleoside amide degradation, but modified nucleic acids are not limited to theses examples. Antisense nucleic acids of the present invention can be preferably designed according to the following principles: increasing the intracellular stability of the nucleic acid, increasing the ability of the nucleic acid to permeate the cell, increasing the affinity of the nucleic acid for the target sense strand, and if the nucleic acid has any toxicity, keeping it as low as possible.

Many such modifications are known in the art, and examples are described in J. Kawakami et al., *Pharm Tech Japan*, vol. 8, pp.247 and pp. 395, 1992; S. T. Crooke et al. ed., *Antisense Research and Applications*, CRC Press, 1993; and the like.

The antisense nucleic acids of the present invention may contain altered or modified sugars, bases or linkages; be delivered in specialized systems such as liposomes or microspheres; be used in gene therapy; or be delivered by additional modes. Moieties that can be used as the additional modes include polycationic moieties such as polylysine that act as charge neutralizers of the phosphate backbone, and hydrophobic [tn 9] moieties such as lipids (e.g., phospholipids, cholesterol, etc.) that enhance interaction with cell membranes and increase uptake of the nucleic acid. Lipids preferred for attachment are cholesterol and cholesterol derivatives (e.g., cholesteryl chloroformate, cholic acid, etc.). The moieties may be attached at the 3' or 5' end of the nucleic acid, and also may be attached through a base, sugar, or internucleoside linkage. Other groups may be capping groups specifically placed at the 3' or 5' ends of the nucleic acids, with examples being groups to inhibit degradation by nuclease such as exonuclease, RNase, etc. Such capping

groups include, but are not limited to, hydroxyl protecting groups known in the art, most importantly glycols such as polyethylene glycols, tetraethylene glycol, and the like.

The inhibitory activity of antisense nucleic acids can be examined using a transformant of the present invention, an in vitro or in vivo gene expression system of the present invention, or an in vitro or in vivo translation system for G protein-coupled receptor proteins. The nucleic acid can be applied to the cell by various methods known per se.

As the DNA encoding the peptide fragment of the present invention, any may be used, provided that it contains the above-described base sequence encoding the peptide fragment of the present invention. Genomic DNA, a genomic DNA library, cDNA derived from the tissues or cells mentioned above, a cDNA library derived from the tissues or cells mentioned above, and synthetic DNA are acceptable. The vector used for a library may be any of bacteriophage, plasmid, cosmid, phagemid, or the like. Furthermore, using an mRNA fraction prepared from the tissues or cells mentioned above, direct amplification can be carried out by RT-PCR.

Specifically, examples of DNA encoding the peptide fragment of the present invention that may be used are (1) DNAs having part of the base sequence of the DNA having the base sequence of SEQ ID NO:2, or (2) DNAs having a base sequence that hybridizes to the base sequence of SEQ ID NO:2 under highly stringent conditions, and a part of the base sequence of a DNA encoding a receptor protein having activity (e.g., ligand binding activity, signal information transducing activity, etc.) substantially the same as that of the receptor protein peptide of the present invention.

As DNA that can hybridize to the base sequence of SEQ ID NO:2, DNA that, for example, contains a base sequence that has homology to the base sequence of SEQ ID NO:2 of at least about 70%, preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% may be used.

Means for cloning a DNA completely encoding the receptor protein or a peptide fragment thereof of the present invention (hereinafter sometimes abbreviated as "receptor protein of the present invention") are PCR amplification using synthetic DNA primers having a partial base sequence of the receptor protein of the present invention, or selection by hybridization of DNA inserted in an appropriate vector with an entity labelled using DNA encoding part or all of the receptor protein of the present invention or synthetic DNA. The hybridization can be performed according to, for example, the method described in *Molecular Cloning*, 2nd (J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989). When a commercially available DNA library is used, the method described in the accompanying instructions can be followed.

Changes in the DNA base sequence can be made using known kits, for example, Mutant ™-G (Takara Shuzo Co.), Mutant ™-K (Takara Shuzo Co.), and the like, by per se known methods such as the Gupped [*sic*] duplex method, Kunkel method, or by an analogous method.

Depending on the purpose, DNA encoding the cloned receptor protein can be used directly, or after digestion with restriction enzymes or addition of linkers, as desired. The DNA may have

ATG as the 5' translation initiation codon, and TAA, TGA, or TAG as the 3' translation termination codon. These translation initiation and termination codons can be added using appropriate synthetic DNA adapters.

An expression vector for the receptor protein of the present invention may be produced, for example, by (a) cutting the desired DNA fragment out of a DNA encoding the receptor protein of the present invention, and (b) attaching the fragment downstream of a promoter in an appropriate expression vector.

Vectors that may be used include plasmids derived from *Escherichia coli* (e.g., pBR322, pBR325, pUC12, pUC13), plasmids derived from *Bacillus subtilis* (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacteriophages such as λ phage, and animal viruses such as retrovirus, vaccinia virus, and baculovirus, as well as other vectors such as pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo, and the like.

In the present invention, any promoter may be used, provided that it is appropriate for the host used for gene expression. For example, when animal cells are used as the host, the SRα promoter, SV40 promoter, LTR promoter, CMV promoter, HSV-TK promoter, or other such promoter may be used.

Among those promoters, the CMV promoter, SRα promoter, and the like are preferable. When the host is bacteria of the genus *Escherichia*, the trp promoter, lac promoter, recA promoter, λ P_L promoter, lpp promoter, etc. are preferable; when the host is bacteria of the genus *Bacillus*, the SPO1 promoter, SPO2 promoter, penP promoter, etc. are preferable; and when the host is a yeast, the PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, etc. are preferable. When the host is an insect cell, polyhedrin promoter, P10 promoter, etc. are preferable.

The expression vectors may further contain desired enhancers, splicing signals, polyadenylation signals, selective markers, SV40 origin of replication (hereinafter sometimes abbreviated as "SV40 ori"), and so on. Examples of selective markers are the dihydrofolate reductase gene (hereinafter sometimes abbreviated as "dhfr"; methotrexate [MTX] resistance), the neomycin resistance gene (hereinafter sometimes abbreviated as Neo^r; G418 resistance), and so on. In particular, desired gene can be selected in thymidine-free medium when the dhfr gene is used as the selective marker in CHO ($dhfr^-$) cells.

When necessary, a signal sequence appropriate for the host is added to the N-terminal side of the receptor protein of the present invention. Signal sequences that can be used include the PhoA signal sequence, the OmpA signal sequence, etc. when the host is bacteria of the genus *Escherichia*; the α-amylase signal sequence, the subtilisin signal sequence, etc. when the host is bacteria of the genus *Bacillus*; the MF α signal sequence, the SUC2 signal sequence, etc. when the host is a yeast; and the insulin signal sequence, the α-interferon signal sequence, the antibody molecule signal sequence, etc. when the host is an animal cell.

Using a vector constructed this way containing DNA encoding the receptor protein of the present invention, a transformant can be produced.

Examples of hosts that may be used include *Escherichia* species, *Bacillus* species, yeast, insect cells, insects, animal cells, and so on.

Examples of *Escherichia* species that can be used include *Escherichia coli* K12 and DH1 (*Proceedings of the National Academy of Sciences of the United States of America*, vol. 60, 160, 1968), JM103 (*Nucleic Acids Research*, vol. 9, 309, 1981), JA221 (*Journal of Molecular Biology*, vol. 120, 517, 1978), HB101 (*Journal of Molecular Biology*, vol. 41, 459, 1969), and C600 (*Genetics*, vol. 39, 440, 1954), and the like.

Examples of *Bacillus* species that can be used include *Bacillus subtilis* MI114 (*Gene*, vol. 24, 255, 1983), 207-21 (*Journal of Biochemistry*, vol. 95, 87, 1984), and the like.

Examples of yeast species that can be used include *Saccharomyces cerevisiae* AH22, AH22R$^-$, NA87-11A, DKD-5D, and 20B-12; *Schizosaccharomyces pombe* NCYC1913 and NCYC2036; *Pichia pastoris*; and the like.

Examples of insect cells that can be used include armyworm larval cell line cells (*Spodoptera frugiperda* cells, Sf cells), MG1 cells derived from the center intestine of *Trichoplusia ni*, High Five ™ cells derived from eggs of *Trichoplusia ni*, cells derived from *Mamestra brassicae*, cells derived from *Estigmena acrea*, etc. when the virus is AcNPV; silkworm cell line cells (*Bombyx mori* N cells, BmN cells) etc. when the virus is BmNPV; and the like. Examples of Sf cells that can be used are Sf9 cells (ATCC CRL 1711), Sf21 cells (for both, Vaughn J. L. et al., *In Vivo*, vol. 13, 213-217, 1977), and the like.

Examples of insects that can be used include silkworm larva (Maeda et al., *Nature*, vol. 315, 592, 1985) and the like.

Examples of animal cells that can be used include COS-7 monkey cells, Vero cells, Chinese hamster CHO cells (hereinafter abbreviated as "CHO cells"), dhfr gene-deficient Chinese hamster CHO cells (hereinafter abbreviated as "CHO [dhfr$^-$] cells"), mouse L cells, mouse AtT-20 cells, mouse myeloma cells, rat GH3 cells, human FL cells, and the like.

Transformation of *Escherichia* species can be performed, for example, according to the methods described in *Proceedings of the National Academy of Sciences of the United States of America*, vol. 69, 2110, 1972; *Gene*, vol. 17, 107, 1982; etc. Transformation of *Bacillus* species can be performed, for example, according to the methods described in *Molecular & General Genetics*, vol. 168, 111, 1979; etc.

Transformation of yeast can be performed, for example, according to the methods described in *Methods in Enzymology*, vol. 194, 182-187, 1991; *Proceedings of the National Academy of Sciences of the United States of America*, vol. 75, 1929, 1978; etc.

Transformation of insect cells or insects can be performed, for example, according to the methods described in *Bio/Technology*, vol. 6, 47-55, 1988; etc.

Transformation of animal cells can be performed, for example, according to the methods described in *Saibo Kogaku Bessatsu 8, Shin Saibo Kogaku Jikken Purotokoru* [translation: Cell

Engineering, Separate Vol. 8, New Cell Engineering Experiment Protocols], 263-267, 1995, Shujun Company, and in *Virology*, vol. 52, 456, 1973.

In these ways, transformants transformed with expression vectors containing DNA encoding the G protein-coupled receptor protein can be obtained.

When culturing transformants in bacterial hosts of the *Escherichia* or *Bacillus* species, liquid culture medium is appropriate, and that medium is caused to contain carbon sources, nitrogen sources, minerals, etc. which are necessary for growing the transformants. Examples of carbon sources include glucose, dextrin, soluble starch, sucrose, and the like. Examples of nitrogen sources include organic and inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, soybean cake, potato extracts, and the like. Examples of minerals include calcium chloride, sodium dihydrogen phosphate, magnesium chloride, and so on. It is further allowable to add yeasts, vitamins, growth-promoting factors, and the like. A culture medium pH of about 5 to 8 is preferable.

A preferable culture medium for *Escherichia* bacteria is, for example, M9 medium containing glucose and casamino acids (Miller, *Journal of Experiments in Molecular Genetics*, 431-433, Cold Spring Harbor Laboratory, New York, 1972. If necessary, drugs such as 3β-indole acrylic acid may be added to increase the efficiency of the promoter. When the host is *Escherichia* bacteria, culture is usually performed at about 15°C to 43°C for about 3 to 24 hours, and aeration and stirring may be added as necessary.

When the host is *Bacillus* bacteria, culture is usually performed at about 30°C to 40°C for about 6 to 24 hours, and aeration and stirring may be added as necessary.

When culturing transformants in yeast hosts, the culture medium may be, for example, Burkholder minimal medium (Bostian, K. L. et al., *Proceedings of the National Academy of Sciences of the United State of America*, vol. 77, 4505, 1980) or SD medium containing 0.5% casamino acids (Bitter, G. A. et al., *Proceedings of the National Academy of Sciences of the United State of America*, vol. 81, 5330, 1984). It is preferable that the pH of the culture medium be adjusted to about 5 to 8. Culture is usually performed at about 20°C to 35°C for about 24 to 72 hours, with aeration and stirring added as necessary.

Culture media that can be used when culturing transformants in insect or insect cell hosts include, for example, Grace's insect medium (Grace, T.C.C., *Nature*, vol. 195, 788, 1962) suitably supplemented with additives such as inactivated 10% bovine serum. It is preferable that the pH of the culture medium be adjusted to about 6.2 to 6.4. Culture is usually performed at about 27°C for about 3 to 5 days, with aeration and stirring added as necessary.

Culture media that can be used when culturing transformants in animal cell hosts include, for example, MEM medium (*Science*, vol. 122, 501, 1952), DMEM medium (*Virology*, vol. 8, 396, 1959), RPMI 1640 medium (*The Journal of the American Medical Association*, vol. 199, 519, 1967), 199 medium (*Proceeding of the Society for the Biological Medicine*, vol. 73, 1, 1950), and the like, with those media containing about 5% to 20% fetal calf serum. It is

preferable that the pH of the culture medium be about 6 to 8. Culture is usually performed at about 30°C to 40°C for about 15 to 60 hours, with aeration and stirring added as necessary.

In the above ways, production of the G protein-coupled receptor protein of the present invention can be caused in the cell membrane of the transformant.

Separation and purification of the receptor protein of the present invention from the above-mentioned cultures can be performed, for example, according to methods the described below.

Methods that may be suitably used to extract the receptor protein of the present invention from the cultured bacteria or [other] cells include methods in which after culture the bacteria or cells are collected by known methods, suspended in an appropriate buffer, and disrupted by such techniques as ultrasonic waves, lysozyme, and/or freezing and thawing, and then a crude receptor protein extract is obtained by centrifugation or filtration. The buffer may contain a protein-denaturing agent such as urea or guanidine hydrochloride, or a surfactant such as Triton X-100 ™. In cases where the receptor protein is secreted into culture media, supernatant is separated from bacteria or cells after completion of culture and is then collected, with the separation and collection being performed by per se known methods.

Purification of the receptor protein from the extract or the culture supernatant obtained this way can be performed by suitably combining per se known methods of separation and purification. Examples of such known methods that can be used include methods utilizing solubility, such as salt precipitation and solvent precipitation; methods mainly utilizing differences in molecular weight, such as dialysis, ultrafiltration, gel filtration, and SDS-polyacrylamide gel electrophoresis; methods utilizing differences in electric charge, such as ion-exchange chromatography; methods utilizing specific affinity, such as affinity chromatography; methods utilizing differences in hydrophobicity, such as reversed-phase high-performance liquid chromatography; methods utilizing differences isoelectric point, such as isoelectric focusing; and the like.

In cases where the receptor protein thus obtained is in a free form, it can be converted to a salt by a per se known method or a method analogous thereto; conversely, when the protein thus obtained is in the form of a salt, it can be converted to the free form or to another salt by a per se known method or a method analogous thereto.

Before or after purification, by causing an appropriate protein-modifying enzyme to act, modifications can be optionally added to the receptor protein produced by the recombinant, or polypeptide can be partly removed [*sic*; tn 10] from that protein. Examples of protein-modifying enzymes that may be used include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, and the like.

The activity of the receptor protein or salt thereof of the present invention thus produced can be measured by binding assay with labelled ligand, enzyme immunoassay using a specific antibody, and the like.

Antibodies to the receptor protein of the present invention, a peptide fragment thereof, or a salt thereof may be either polyclonal or monoclonal, provided that they are capable of

recognizing the receptor protein of the present invention, a peptide fragment thereof, or a salt thereof.

Antibodies to the receptor protein of the present invention, a peptide fragment thereof, or a salt thereof (hereinafter sometimes abbreviated as "receptor protein of the present invention") may be produced by per se known methods of producing antibodies or antisera, using the receptor protein of the present invention as an antigen.

Preparation of Monoclonal Antibodies

(a) Preparation of Monoclonal Antibody-Producing Cells

The receptor protein of the present invention is administered to a mammal, either alone or together with carrier or diluent, to a site that allows antibody production after such administration. At the administration, Freund complete or incomplete adjuvant may also be given to increase antibody production capability. Usually a total of about 2 to 10 administrations are performed at a frequency of one every 2 to 6 weeks. Examples of mammals that may be used include monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, and goats, with mice and rats being preferred.

Monoclonal antibody-producing cells can be prepared by selecting an individual with a detectable antibody titer from warm-blooded animals, for example mice, immunized with the antigen, collecting the spleen or lymph nodes from the animal 2 to 5 days after the final immunization, and fusing antibody-producing cells from the spleen or lymph nodes with myeloma cells, which results in the production of monoclonal antibody-producing hybridomas. The antibody titer in antiserum can be measured, for example, by reacting labelled receptor protein, which will be mentioned below, with antiserum and then measuring the activity of the antibody-bound labelling agent. The [cell] fusion may be performed by a known method, such as that of Kohler and Milstein (*Nature*, vol. 256, page 495, 1975). Examples fusion-promoting agents include polyethylene glycol (PEG), Sendai virus, and the like, with the use of PEG being preferred.

Examples of the myeloma cells include NS-1, P3U1, SP2/0, and the like, with the use of P3U1 being preferred. Preferable ratios of number of antibody-producing cells (spleen cells) to number of myeloma cells are about 1:1 to 1:20. Efficient cell fusion can be accomplished by adding PEG (preferably PEG 1000 to PEG 6000) to a concentration of 10% to 80%, and then incubating at about 20°C to 40°C, preferably about 30°C to 37°C, for about 1 to 10 minutes.

A variety of methods may be used for screening for hybridomas that produce monoclonal antibodies. For example, in one method a hybridoma culture supernatant is added to a solid phase (for example, microplate) to which the antigen (receptor protein of the present invention) has been adsorbed either directly or with a carrier; then monoclonal antibodies binding to the solid phase are detected by addition of anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody when mouse cells are used for cell fusion) or protein A labelled with radioactive material, enzyme, or the like. In another method, hybridoma culture supernatant is

added to a solid phase to which anti-immunoglobulin antibody or protein A has been adsorbed either directly or with a carrier; then monoclonal antibodies binding to the solid phase are detected by addition of receptor protein labelled with radioactive material, enzyme, or the like.

Selection of monoclonal antibodies may be performed by a per se known method or a method analogous thereto, and usually may be performed, for example, in animal cell culture medium to which HAT (hypoxanthine, aminopterin and thymidine) has been added. Any medium may be used as the medium for selection and growth, provided that the hybridomas can grow in it. Examples of media that can be used include RPMI 1640 medium (Wako Pure Chemicals Industries) containing 1% to 20%, preferably 10% to 20%, fetal calf serum; GIT medium (Wako Pure Chemicals Industries) containing 1% to 10% fetal calf serum; SFM-101 serum-free medium for hybridoma culture (Nissui Pharmaceutical Co.); and the like. The temperature for culture is usually 20°C to 40°C, and preferably is about 37°C. The culture period is usually 5 days to 3 weeks, with 1 to 2 weeks being preferred. Culture normally can be carried out under 5% carbon dioxide.

The antibody titer in the hybridoma culture supernatant can be measured in the same manner as the antibody titer in antiserum described above.

(b) Purification of the Monoclonal Antibodies

Like ordinary separation and purification of polyclonal antibodies, the separation and purification of monoclonal antibodies may be performed by methods of separating and purifying immunoglobulins (for example, salt precipitation, alcohol precipitation, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers [e.g., DEAE], ultracentrifugation, gel filtration, and specific methods of purification in which by using an active adsorbent such as protein A, protein G, or an antigen-bound solid phase, only antibodies are collected, and then those antibodies are obtained by dissociation of the bonds).

Preparation of Polyclonal Antibodies

Polyclonal antibodies of the present invention can be produced by per se known methods or methods analogous thereto. For example, such polyclonal antibodies can be produced by preparing a complex of immunogen (receptor protein antigen) and carrier protein, immunizing a warm-blooded animal in the same manner as described above for the production of monoclonal antibodies, harvesting material containing antibodies to the receptor protein of the present invention from the immunized animal, and separating and purifying the antibodies.

In regard to immunogen-carrier protein complexes for use in immunizing the warm-blooded animal, any type of carrier protein, and any ratio of carrier and hapten crosslinked to it, are acceptable, provided that production of antibodies to the hapten that is crosslinked to the carrier and used for immunization proceeds efficiently; for example, a method in which bovine serum albumin, bovine thyroglobulin, keyhole limpet hemocyanin, or the like is coupled to hapten in a carrier-to-hapten weight ratio of about 0.1:1 to 20:1, preferably about 1:1 to 5:1, may be used.

A variety of condensing agents can be used for the coupling of the hapten and carrier; for example, a glutaraldehyde, carbodiimide, maleimide, or a thiol or dithiopyridyl group-containing active ester reagent can be employed.

The condensation reaction product is administered to a warm-blooded animal at a site that allows antibody production after such administration. At the administration, Freund complete or incomplete adjuvant may also be given to increase antibody production capability. Usually a total of about 3 to 10 administrations are performed at a frequency of one every 2 to 6 weeks.

Polyclonal antibodies can be harvested from blood, ascites, or other body fluid, preferably from blood, of the warm-blooded animal immunized as described above.

The polyclonal antibody titer in the antiserum can be measured in a manner the same as that described above for the antibody titer in serum. Separation and purification of polyclonal antibodies can be performed by methods of separating and purifying immunoglobulins the same as the methods described above for separation and purification of monoclonal antibodies.

The receptor protein or salts thereof, peptide fragments of the protein or salts thereof, and DNA encoding the protein or fragments of the protein of the present invention can be used (1) in determining ligands (agonists) for the G protein-coupled receptor protein of the present invention; (2) as drugs for preventing and/or treating diseases related to insufficient function of the G protein-coupled receptor protein of the present invention; (3) as genetic diagnostic agents; (4) in methods of screening for compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the present invention; (5) as drugs for preventing and/or treating various diseases, those drugs containing compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the present invention; (6) in methods of quantifying ligands for the G protein-coupled receptor protein of the present invention; (7) in methods of screening for compounds (agonists, antagonists, etc.) that alter the binding of ligands to the G protein-coupled receptor protein of the present invention; (8) as drugs for preventing and/or treating various diseases, those drugs containing compounds (agonists, antagonists) that alter the binding of ligands to the G protein-coupled receptor protein of the present invention; (9) in quantifying the receptor protein of the present invention, peptide fragments thereof, and salts thereof; (10) in methods of screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane; (11) as drugs for preventing or treating various diseases, those drugs containing compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane; (12) in neutralization by antibodies to the receptor protein, peptide fragments thereof, or salts thereof of the present invention; (13) in the creation of nonhuman animals having DNA encoding the receptor protein of the present invention; and so on.

In particular, by using a receptor-binding assay system employing an expression system for recombinant G protein-coupled receptor protein of the present invention, it is possible to screen for compounds that alter the binding of ligands (e.g., agonists, antagonists, etc.) specific for the G protein-coupled receptor protein in humans and [other] mammals, and the agonists or

antagonists can be used, for example, as drugs for the prevention or treatment of various diseases.

Below, uses will be specifically explained for the receptor protein, peptide fragments thereof, or salts thereof of the present invention (hereinafter sometimes abbreviated as "receptor protein of the present invention"), DNA encoding the receptor protein or peptide fragments thereof of the present invention (hereinafter sometimes abbreviated as "DNA of the present invention"), and antibodies to the receptor protein of the present invention (hereinafter sometimes abbreviated as "antibodies of the present invention").

(1) Determining of Ligands (Agonists) for the G Protein-coupled Receptor Protein of the Present Invention

The receptor protein or its salts of the present invention, or the peptide fragments or their salts of the present invention, are useful as reagents for searching for, or determining, ligands (agonists) for the receptor protein or salts thereof of the present invention.

That is, the invention provides a method of determining ligands to the receptor protein of the present invention that is characterized by bringing the receptor protein or salt thereof of the present invention, or a peptide fragment or salt thereof of the present invention, into contact with a test compound.

Examples of test compounds that can be used include tissue extracts, cell culture supernatants, and the like from humans or [other] mammals (mice, rats, pigs, bovines, sheep, monkeys, etc.), as well as known ligands (for example, angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP [vasoactive intestinal and related polypeptide], somatostatin [*sic*], dopamine, motilin, amylin, bradykinin, CGRP [calcitonin gene-related peptide], leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, α- and β-chemokines [e.g., IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.], endothelin, enterogastrin [*sic*], histamine, neurotensin, TRH, pancreatic polypeptide, galanin, etc.). For example, by adding tissue extract, cell culture supernatant, or the like to the receptor protein of the present invention and measuring cell stimulation activity etc. while performing fractionation, a single ligand can finally be obtained.

Specifically, ligand determination methods of the present invention are methods in which by using the receptor protein of the present invention, peptide fragments of the receptor protein, or salts thereof, or by constructing an expression system for recombinant receptor protein and using a receptor-binding assay system employing that expression system, compounds (e.g., peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, etc.) or their salts, which upon binding the receptor protein of the present invention have cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) are determined.

The ligand determination methods of the present invention are characterized by measuring, for example, the cell stimulation activity, or the amount of test compound bound to the receptor protein or peptide fragment thereof of the present invention, when the receptor protein or peptide fragment is brought into contact with the test compound.

More specifically, the present invention provides:

① A method of determining ligands for the receptor protein or a salt thereof of the present invention which is characterized by measuring the amount of a labelled test compound bound

to the receptor protein or a salt thereof of the present invention, or to a peptide fragment or a salt thereof of the present invention, when the labelled test compound has been brought into contact with the receptor protein or salt thereof, or with the peptide fragment or salt thereof;

② A method of determining ligands for the receptor protein or a salt thereof of the present invention which is characterized by measuring the amount of a labelled test compound bound to cells containing the receptor protein of the present invention, or to the membrane fraction of such cells, when the labelled test compound has been brought into contact with the cells or membrane fraction;

③ A method of determining ligands for the receptor protein of the present invention which is characterized by measuring the amount of a labelled test compound bound to the receptor protein or a salt thereof of the present invention when the labelled test compound has been brought into contact with the receptor protein expressed on the cell membrane by culturing a transformant containing DNA encoding the receptor protein;

④ A method of determining ligands for the receptor protein or a salt thereof of the present invention which is characterized by measuring cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) mediated by the receptor protein of the present invention when a test compound has been brought into contact with cells containing the receptor protein; and

⑤ A method of determining ligands for the receptor protein or a salt thereof of the present invention which is characterized by measuring cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) mediated by the receptor protein of the present invention when a test compound is brought into contact with the receptor protein expressed on the cell membrane by culturing a transformant containing DNA encoding the receptor protein.

In particular, it is preferable to confirm that the test compound binds to the receptor protein of the present invention by the tests of ① to ③ above, and then to perform the tests of ④ and ⑤ above.

The receptor protein used for ligand determination may be any protein provided that it contains the above-described receptor protein of the present invention or peptide fragment of the present invention; [in particular], receptor protein expressed in large quantity by the use of animal cells is suitable.

For production of the receptor protein of the present invention, the method of expression described above can be used, and carrying out production of the protein by expressing DNA encoding the receptor protein in cells of mammals or insects is preferable. Normally, cDNA is used as the DNA fragment encoding the desired part of the protein, but there is no necessary limitation to cDNA. For example, a gene fragment or a synthetic DNA may also be used. For transferring the DNA fragment encoding the receptor protein of the present invention into a host animal cell and expressing it efficiently, it is preferable to insert the DNA fragment downstream of a promoter such as the polyhedrin promoter of nuclear polyhedrosis virus (NPV), which is a baculovirus with insect hosts, a promoter derived from SV40, a retroviral promoter, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SRα promoter, or the like. Quantitative and qualitative assay of the expressed receptor protein can be carried out by per se known methods. For example, the method described in Nambi, P. et al., *The Journal of Biological Chemistry*, vol. 267, page 19,555-19,559, 1992, may be used.

Therefore, the entity containing the receptor protein, a peptide fragment of the protein, or a salt thereof of the present invention in the ligand determination of the invention may be purified receptor protein, peptide fragment of the protein, or salt thereof purified according to a per se known method, or it may be cells containing the receptor protein or the membrane fraction of such cells.

When cells containing the receptor protein of the present invention are used in the ligand determination of the invention, the cells may be fixed in glutaraldehyde, formalin, or the like. Fixation can be performed by a per se known method.

The cells containing the receptor protein of the present invention are host cells expressing the receptor protein of the invention. *Escherichia coli*, *Bacillus subtilis*, yeast, insect cells, animal cells, and the like may be used as the host cells.

A cell membrane fraction is a fraction containing a large amount of cell membranes obtained by a per se known method after disruption of the cells. Examples of methods of cell disruption include crushing in a Potter-Elvehjem homogenizer, disruption in a Waring blender or Polytron (Kinematica), disruption by ultrasonication, disruption by squirting through a narrow nozzle while applying pressure with a French press etc., and the like. For fractionation of the cell membranes, methods employing centrifugal force such as fractionation centrifugation, density-gradient centrifugation, and the like are mainly used. For example, a suspension of disrupted cells is centrifuged at low speed (500 to 3000 rpm) for a short period of time (normally, about 1 to 10 minutes); the supernatant is then further centrifuged at high speed (15,000 to 30,000 rpm), normally for 30 minutes to 2 hours, and the sediment obtained is regarded as membrane fraction. This cell membrane fraction contains the expressed receptor protein and many membrane components, such as phospholipids, membrane proteins, and the like.

The amount of receptor protein in cells containing the receptor protein, and in the membrane fraction from such cells, is preferably 10^3 to 10^8 molecules per cell, and more preferably 10^5 to 10^7 molecules per cell. At higher levels of expression, ligand binding activity (specific activity)

of the membrane fraction is increased, which makes possible not only the construction of a highly sensitive screening system, but also determination of a large number of samples in one lot.

To carry out methods ① to ③ above of determining ligands for the receptor protein or salt thereof of the present invention, a suitable receptor protein fraction and labelled test compound are necessary.

The receptor protein fraction is preferably a naturally occurring receptor protein fraction, a recombinant receptor protein fraction having activity equivalent to that, or the like. Used herein, the term "equivalent activity" means equivalent ligand binding activity, signal information transducing activity, and the like.

The labelled test compound is preferably angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptide), somatostatin [*sic*], dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, α- and β-chemokines (e.g., IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enterogastrin [*sic*], histamine, neurotensin, TRH, pancreatic polypeptide, galanin, or the like labelled with [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc.

Specifically, to carry out a method of determining ligands for the receptor protein or salt thereof of the present invention, a receptor sample is first prepared by suspending the cells or cell membrane fraction containing the receptor protein of the present invention in a buffer appropriate for the determination method. Any buffer that does not interfere with binding of the ligand to the receptor protein can be used, for example, a phosphate buffer, Tris-HCl buffer, or the like of pH 4 to 10 (preferably pH 6 to 8). To decrease nonspecific binding, a surfactant such as CHAPS, Tween 80 ™ (Kao-Atlas), digitonin, deoxycholate, or the like, and various proteins such as bovine serum albumin, gelatin, and the like, can be added to the buffer. In addition, to inhibit degradation of the receptor protein and ligand by proteases, a protease inhibitor such as PMSF, leupeptin, E-64 (Peptide Institute), pepstatin, or the like can also be added to the buffer. A given amount (5000 to 500,000 cpm) of the test compound labelled with [^{3}H], [^{125}I], [^{14}C], [^{35}S], or the like is added to 0.01 to 10 ml of receptor suspension. To determine the amount of nonspecific binding, a reaction tube with a large excess of unlabelled test compound is also prepared. The reaction is carried out at about 0°C to 50°C, preferably about 4°C to 37°C, for about 20 minutes to 24 hours, preferably about 30 minutes to 3 hours. After the reaction, [the reaction mixture] is filtered through a glass fiber filter or the like; then, after [the filter] has been washed with a suitable amount of the same buffer, the radioactivity remaining on the filter is measured using a liquid scintillation counter or γ-counter. Test compounds whose count (B – NSB) obtained by subtracting nonspecific binding (NSB) from total binding (B) exceeds 0 cpm

can be selected as ligands (agonists) for the receptor protein or salt thereof of the present invention.

To carry out methods ④ and ⑤ above of determining ligands for the receptor protein or salt thereof of the present invention, stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) mediated by the receptor protein of the present invention can be measured by a known method or by a commercially available kit. Specifically, the cells containing the receptor protein are first cultured in a multiwell plate or the like. At the time of ligand determination, before addition of the test compound, [the culture medium] is replaced with fresh medium or with an appropriate buffer that is not toxic to the cells. Then test compound etc. is added and the culture is incubated for a given period of time. After the incubation, the cells are extracted or the supernatant is recovered, and the products formed are quantified by suitable methods. When assay of the formation of the substance (e.g., arachidonic acid, etc.) serving as an index of cell stimulation activity is difficult because of a degradative enzyme in the cells, the assay can be performed after addition of an inhibitor of the degradative enzyme. In addition, cAMP production-inhibiting activity and the like can be detected as inhibition of production in cells whose basal production has been increased by forskolin, etc.

A kit for determining ligands that bind to the receptor protein or a salt thereof of the present invention contains the receptor protein or a salt thereof of the present invention, a peptide fragment or a salt thereof of the present invention, cells containing the receptor protein of the present invention, a membrane fraction of cells containing the receptor protein of the present invention, or the like.

The following are examples of a kit for ligand determination of the present invention.

1. Reagents for Ligand Determination

① Measurement buffer and wash buffer

Hanks' balanced salt solution (Gibco) with 0.05% bovine serum albumin (Sigma) added to it.

The buffer is either sterilized by filtration through a filter with a pore diameter of 0.45 μm and then stored at 4°C, or prepared at the time of use.

② G protein-coupled receptor protein sample

CHO cells expressing the receptor protein of the present invention passaged into 12-well plates at 5 x 10^5 cells/well, and cultured at 37°C, in 5% CO_2 and 95% air, for 2 days.

③ Labelled test compound

A compound labelled with commercially available [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc., or a compound labelled by an appropriate method.

An aqueous solution of labelled test compound is stored at 4°C or -20°C, and when it is used, it is diluted to 1 μM with measurement buffer. Test compounds that are insoluble in water are dissolved in dimethylformamide, DMSO, methanol, and the like.

④ Unlabelled test compound

Unlabelled compound the same as the labelled compound is prepared at a concentration 100 to 1000 times greater than that of the labelled test compound.

2. Measurement

① CHO cells expressing the receptor protein of the present invention cultivated in a 12-well tissue culture plate are washed twice with 1 ml of measurement buffer. Then 490 μl of measurement buffer is added to each well.

② Labelled test compound 5 μl is added, and the reaction is allowed to proceed at room temperature for one hour. To determine the amount nonspecific binding, 5 μl of unlabelled test compound is added.

③ The reaction mixture is removed, and washing with 1 ml of wash buffer is done 3 times. Labelled test compound bound to the cells is dissolved with 0.2-N NaOH-1% SDS and mixed with 4 ml of liquid scintillator A (Wako Pure Chemicals Industries).

④ Radioactivity is measured with a liquid scintillation counter (Beckman).

Ligands that can bind to the receptor protein or a salt thereof of the present invention include, for example, substances specifically present in brain, pituitary, pancreas, etc. Examples of substances that can be used include angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptide), somatostatin [*sic*], dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, α- and β-chemokines (e.g., IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enterogastrin [*sic*], histamine, neurotensin, TRH, pancreatic polypeptide, galanin, and so on.

(2) Drugs for Preventing and/or Treating Diseases Related to Insufficient Function of the G Protein-coupled Receptor Protein of the Present Invention

If a ligand for the receptor protein of the present invention is found by the method of section (1) above, according to the action of the ligand ① the receptor protein of the present invention, or ② DNA encoding the receptor protein can be used as a drug etc. for preventing and/or treating diseases related to insufficient function of the G protein-coupled receptor protein of the present invention. For example, in a patient in whom the physiological effect of the ligand cannot be

expected because the receptor protein of the present invention is decreased in the body (deficiency of receptor protein), the level of the receptor protein in the body can be increased so that the ligand has sufficient effect by, for example, ① administering the receptor protein to the patient to increase its level, or ② (a) administering DNA encoding the receptor protein to the patient and thereby causing the protein to be expressed in the patient, or (b) inserting DNA encoding the receptor protein into the target cells, expressing the protein, and then transplanting the cells in the patient. Thus, DNA encoding the receptor protein of the present invention is useful as a safe, low-toxicity drug for preventing and/or treating diseases related to insufficient function of the receptor protein of the present invention.

At the amino acid sequence level, the receptor protein of the present invention has homology of about 60% to the SLC-1 receptor and to somatostatin receptor type 3 (SS3R) and type 5 (SS5R), which are G protein-coupled receptor proteins.

The receptor protein of the present invention and DNA encoding the receptor protein of the present invention are useful as drugs for preventing and/or treating central diseases (e.g., Alzheimer disease, dementia, eating disorders [cibophobia], epilepsy, etc.), hormone system diseases [*sic*; tn 11] (e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.), hepatic/biliary/pancreatic/endocrine diseases (e.g., diabetes, eating disorders, etc.), inflammatory diseases (e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases (e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.).

When the receptor protein of the present invention is to be used as a preventive or therapeutic drug as described above, the protein can be formulated by conventional means.

When DNA encoding the receptor protein of the present invention (hereinafter sometimes abbreviated as "DNA of the present invention") is to be used as a preventive or therapeutic drug as described above, the DNA of the invention can be administered according to a conventional method either alone, or after insertion into a suitable vector such as a retroviral vector, adenoviral vector, adenovirus associated viral vector, or the like. The DNA of the present invention can be administered alone, or with adjuvant to facilitate uptake, by means of a gene gun or a catheter such as a hydrogel catheter.

For example, ① the receptor protein of the present invention, or ② DNA encoding the receptor protein may be used orally in the form of tablets that may be sugar coated as necessary, capsules, elixirs, microcapsules, and the like, or parenterally in the form of injectable preparations such as sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. These preparations can be produced, for example, by mixing ① the receptor protein of the present invention, or ② DNA encoding the receptor protein, with physiologically acceptable known carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, binders, and the like in unit dosage forms required for generally accepted pharmaceutical practice. The amount of active ingredient in these preparations is set so that an appropriate dose within the specified range is obtained.

Additives that can be incorporated in tablets, capsules, and the like include, for example, binders such as gelatin, corn starch, tragacanth, and gum arabic; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose, and saccharin; and flavoring agents such as peppermint, akamono oil, and cherry. When the unit dosage form is a capsule, the above-mentioned materials may also include liquid carriers such as oils and fats. Sterile compositions for injection can be formulated according to ordinary pharmaceutical practice such as by dissolving or suspending the active ingredient and naturally occurring vegetable oils such as sesame oil, coconut oil, and the like in a vehicle such as water for injection. Aqueous liquids for injection that may be used include, for example, physiological saline and isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride), and the aqueous liquids may be used in combination with appropriate solubilizing agents such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80 ™, HCO-50), and the like. Oily liquids that may be used include, for example, sesame oil and soybean oil, and the oily liquids may be used in combination with solubilizing agents such as benzyl benzoate and benzyl alcohol.

Furthermore, the preventive or therapeutic drugs described above may also be combined with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, and the like. The prepared solution for injection is normally packaged in an appropriate ampule.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the receptor protein of the present invention for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the DNA of the present invention for oral administration is, for example, in cibophobia patients (weighing 60 kg), about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the

subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(3) Genetic Diagnostic Agents

By using the DNA of the present invention as a probe, abnormalities can be detected in the DNA or mRNA encoding the receptor protein of the present invention or a peptide fragment thereof (genetic abnormalities) in humans or [other] mammals (e.g. rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.). Therefore, the DNA of the present invention is useful, for example, as a genetic diagnostic agent for damage to the DNA or RNA, mutations in the DNA or RNA, and decreased expression of the DNA [*sic*] or RNA, as well as for increased levels of the DNA or RNA, or overexpression of the DNA or RNA.

The above-mentioned genetic diagnosis using the DNA of the present invention can be performed by, for example, the per se known Northern hybridization assay or PCR-SSCP assay (*Genomics*, vol. 5, page 874-879, 1989, and *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, page 2766-2770, 1989).

(4) Methods of Screening for Compounds That Alter the Level of Expression of the Receptor Protein or Peptide Fragments Thereof of the Present Invention

The DNA of the present invention can be used as a probe in screening for compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the present invention.

That is, the present invention provides, for example, a method of screening for compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the present invention by measuring the amount of mRNA for the receptor protein or a peptide fragment thereof of the present invention contained in (i) ① blood, ② specific organs, or ③ tissues or cells isolated from organs, of nonhuman mammals, or (ii) transformants, or the like.

Specifically, measurement of the amount of mRNA for the receptor protein or a peptide fragment thereof of the present invention is performed as described below.

(i) Normal or disease model nonhuman mammals (e.g., mice, rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.; more specifically demented rats, obese mice, arteriosclerotic rabbits, tumor-bearing mice, etc.) are subjected to drugs (e.g., antidementia agents, hypotensive agents, antineoplastic agents, antiobesity agents, etc.), physical stress (e.g., water immersion stress, electric shock, light/darkness, low temperature, etc.), or the like, and after a fixed period of time, blood, specific organs (e.g., brain, liver, kidney, etc.), or tissue or cells isolated from organs, are obtained.

Messenger RNA for the receptor protein or a peptide fragment thereof of the present invention in the cells that are obtained, for example, can be extracted from the cells etc. by an ordinary method, and quantified, for example, by a technique such as Taq Man PCR or the like, and the mRNA can be analyzed by performing Northern blotting by a per se known means.

(ii) A transformant expressing the receptor protein or a peptide fragment thereof of the present invention is prepared according to the method described above. Then mRNA for the

receptor protein or peptide fragment thereof contained in the transformant can be quantified and analyzed in the same way.

Screening for compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the present invention can be performed by,

(i) Administering a test compound to normal or disease model nonhuman mammals at a fixed period of time before (30 minutes to 24 hours before, preferably 30 minutes to 12 hours before, more preferably 1 hour to 6 hours before), a fixed period of time after (30 minutes to 3 days after, preferably 1 hour to 2 days after, more preferably 1 hour to 24 hours after), or at the same time as the animals are subjected to drugs or physical stress, and quantifying and analyzing the amount of mRNA of the receptor protein or peptide fragment thereof of the invention contained in cells at a fixed period of time after (30 minutes to 3 days after, preferably 1 hour to 2 days after, more preferably 1 hour to 24 hours after) administration; and

(ii) Adding a test compound to the culture medium when culturing a transformant by an ordinary method; then after (1 to 7 days after, preferably 1 to 3 days after, more preferably 2 to 3 days after) culture for a fixed period of time, quantifying and analyzing the amount of mRNA for the receptor protein or peptide fragment thereof of the present invention contained in the transformant.

Compounds or salts thereof obtained by using the screening method of the present invention are compounds that have the action of altering the level of expression of the receptor protein or a peptide fragment thereof of the present invention; specifically, (a) compounds that increase G protein-coupled receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) by increasing the level of expression of the receptor protein or a peptide fragment thereof of the present invention; and (b) compounds that decrease the cell stimulation activity by decreasing the level of expression of the receptor protein or a peptide fragment thereof of the present invention. Examples of the compounds include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, and the like. These compounds may be novel or known.

Compounds that increase the cell stimulation activity are useful as safe drugs of low toxicity (for example, drugs for preventing and/or treating central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.]) that increase the physiological activity of the receptor protein of the present invention.

Compounds that decrease the cell stimulation activity are useful as safe drugs of low toxicity that decrease the physiological activity of the receptor protein of the present invention.

[0067]

When a compound or salt thereof obtained using the screening method of the present invention is used as a pharmaceutical composition, [the compostion] can be practiced by conventional means. For example, in the same way as drugs containing the receptor protein of the present invention described above, [the composition] may be a tablet, capsule, elixir, microcapsule, sterile solution, suspension, or the like.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(5) Drugs Containing Compounds that Alter the Level of Expression of the Receptor Protein or Peptide Fragments Thereof of the Present Invention for Preventing and/or Treating Various Diseases

As explained above, the receptor protein of the present invention is thought to play some important role in the body, for example a central function. Therefore, compounds that alter the level of expression of the receptor protein or peptide fragments thereof of the invention can be used as drugs for preventing and/or treating diseases related to insufficient function of the receptor protein (for example, central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.] etc.).

When such a compound is used as a drug for preventing and/or treating diseases related to insufficient function of the receptor protein of the present invention, [the compound] can be formulated by conventional means.

For example, the compound may be used orally in the form of tablets that may be sugar coated as necessary, capsules, elixirs, microcapsules, and the like, or parenterally in the form of injectable preparations such as sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. These preparations can be produced, for example, by mixing the compound with physiologically acceptable known carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, binders, and the like in unit dosage forms required for generally accepted pharmaceutical practice. The amount of active ingredient in these preparations is set so that an appropriate dose within the specified range is obtained.

Additives that can be incorporated in tablets, capsules, and the like include, for example, binders such as gelatin, corn starch, tragacanth, and gum arabic; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose, and saccharin; and flavoring agents such as peppermint, akamono oil, and cherry. When the unit dosage form is a capsule, the above-mentioned materials may also include liquid carriers such as oils and fats. Sterile compositions for injection can be formulated according to ordinary pharmaceutical practice such as by dissolving or suspending the active ingredient and naturally occurring vegetable oils such as sesame oil, coconut oil, and the like in a vehicle such as water for injection. Aqueous liquids for injection that may be used include, for example, physiological saline and isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride), and the aqueous liquids may be used in combination with appropriate solubilizing agents such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80 ™, HCO-50), and the like. Oily liquids that may be used include, for example, sesame oil and soybean oil, and the oily liquids may be used in combination with solubilizing agents such as benzyl benzoate and benzyl alcohol.

Furthermore, the preventive or therapeutic drugs described above may also be combined with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, and the like. The prepared solution for injection is normally packaged in an appropriate ampule.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral

administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(6) Methods of Quantifying Ligands for the G Protein-coupled Receptor Protein of the Present Invention

Because the receptor protein of the present invention has affinity for ligands, it can quantify ligand concentrations in the body with good sensitivity.

The quantification method of the present invention, for example, may be used in combination with a competition method. The ligand concentration in the sample to be quantified can be measured by bringing the sample into contact with the receptor protein of the invention. Specifically, for example, [the receptor protein] can be used according to the methods described in ① or ② below, or the like, or analogous methods.

① Hiroshi Irie (ed): *Rajioimunoassei* [translation: Radioimmunoassay] (Kodansha, 1974)
② Hiroshi Irie (ed): *Zoku Rajioimunoassei* [translation: Radioimmunoassay Continued] (Kodansha, 1979).

(7) Methods of Screening for Compounds (Agonists, Antagonists, Etc.) That Alter the Binding of Ligands to the G Protein-coupled Receptor Protein of the Present Invention

By using the receptor protein of the present invention, or by constructing an expression system for recombinant receptor protein and using a receptor-binding assay system employing that expression system, screening for compounds (e.g., peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, etc.) or salts thereof that alter the binding of ligands to the receptor protein of the present invention can be carried out efficiently.

Examples of such compounds include (a) compounds that have G protein-coupled receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) (so-called "agonists" of the receptor protein of the present invention), (b) compounds that do not have said cell stimulation activity (so-called "antagonists" of the receptor protein of the present invention), (c) compounds that increase the binding strength between ligands and the G protein-coupled receptor protein of the present invention, and (d) compounds that decrease the binding strength between ligands and the G protein-coupled receptor protein of the present invention (for

compounds of [a] above, screening by the ligand determination methods described above is preferable).

That is, the present invention provides a method of screening for compound or salts thereof that alter the binding of ligands to the receptor protein, peptide fragments thereof, or salts thereof of the present invention, said method being characterized by comparing (i) the case in which the receptor protein, a peptide fragment thereof, or a salt thereof of the present invention is brought into contact with a ligand, and (ii) the case in which the receptor protein, a peptide fragment thereof, or a salt thereof of the present invention is brought into contact with the ligand and a test compound.

The screening method of the present invention is characterized by measuring and comparing, for example, cell stimulation activity, amount of ligand bound to the receptor protein, and the like in (i) and (ii).

More specifically, the present invention provides:

① A method of screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention which is characterized by measuring and comparing the amount of binding of a labelled ligand to the receptor protein when the labelled ligand has been brought into contact with the receptor protein, and when the labelled ligand and a test compound have been brought into contact with the receptor protein;

② A method of screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention which is characterized by measuring and comparing the amount of binding of a labelled ligand to cells containing the receptor protein of the present invention, or to the membrane fraction of such cells, when the labelled ligand has been brought into contact with the cells or the membrane fraction, and when the labelled ligand and a test compound have been brought into contact with the cells or the membrane fraction;

③ A method of screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention which is characterized by measuring and comparing the amount of binding of a labelled ligand to the receptor protein when the labelled ligand has been brought into contact with the receptor protein expressed on the cell membrane by culturing a transformant containing the DNA of the present invention, and when the labelled ligand and a test compound have been brought into contact with receptor protein expressed on the cell membrane by culturing a transformant containing the DNA of the present invention;

④ A method of screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention which is characterized by measuring and comparing receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) when a compound that activates the receptor protein of the present invention

(for example, a ligand etc. for the receptor protein) has been brought into contact with cells containing the receptor protein, and when a test compound and the compound that activates the receptor protein have been brought into contact with cells containing the receptor protein; and

⑤ A method of screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention which is characterized by measuring and comparing receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) when a compound that activates the receptor protein (for example, a ligand etc. for the receptor protein) has been brought into contact with the receptor protein expressed on the cell membrane by culturing a transformant containing DNA of the present invention, and when a test compound and the compound that activates the receptor protein have been brought into contact with the receptor protein expressed on the cell membrane by culturing a transformant containing DNA of the present invention.

Before the receptor protein of the present invention is [*sic*; was] obtained, when screening for agonists and antagonists of G protein-coupled receptors it was necessary first to obtain candidate compounds by using cells, tissues, or a cell membrane fraction thereof containing G protein-coupled receptor protein of rats etc. (primary screening), and then to perform tests to determine whether the candidate compounds actually inhibited the binding of ligands to human G protein-coupled receptor protein (secondary screening). When cells, tissues, or a cell membrane fraction thereof are used as such, screening for agonists or antagonists of a certain receptor protein is difficult because other receptor proteins are also present.

However, for example, if the receptor protein of human origin of the present invention is used, primary screening is not necessary, and screening for compounds that inhibit the binding of a ligand to the G-protein coupled receptor protein can be performed efficiently. In addition, whether the screened compound is an agonist or antagonist can be readily determined.

The screening method of the present invention is explained in detail below.

The receptor protein of the present invention used in the screening method of the present invention may be any provided that it contains the above-described receptor protein of the present invention. A cell membrane fraction of a mammalian organ containing the receptor protein of the present invention is suitable. However, because organs of human origin are extremely difficult to obtain, receptor protein of rat origin expressed in large amounts using a recombinant, and the like, are suitable for use in screening.

For production of the receptor protein of the present invention, the method described above can be used, and carrying out production of the protein by expressing DNA of the present invention in cells of mammals or insects is preferable. As the DNA fragment encoding the

desired part of the protein, cDNA is used, but there is no necessary limitation to cDNA. For example, a gene fragment or a synthetic DNA may also be used. For transferring the DNA fragment encoding the receptor protein of the present invention into a host animal cell and expressing it efficiently, it is preferable to insert the DNA fragment downstream of a promoter such as the polyhedrin promoter of nuclear polyhedrosis virus (NPV), which is a baculovirus with insect hosts, a promoter derived from SV40, a retroviral promoter, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SRα promoter, or the like. Quantitative and qualitative assay of the expressed receptor protein can be carried out by per se known methods. For example, the method described in Nambi, P. et al., *The Journal of Biological Chemistry*, vol. 267, page 19,555-19,559, 1992, may be used.

Therefore, in the screening method of the present invention, the entity containing the receptor protein of the present invention may be receptor protein purified according to a per se known method; it is also acceptable to use cells containing the receptor protein, or to use the membrane fraction of cells containing the receptor protein.

When cells containing the receptor protein of the present invention are used in the screening method of the invention, the cells may be fixed in glutaraldehyde, formalin, or the like. Fixation can be performed by a per se known method.

The cells containing the receptor protein of the present invention are host cells expressing the receptor protein. Preferable host cells include *Escherichia coli*, *Bacillus subtilis*, yeast, insect cells, animal cells, and the like.

A cell membrane fraction is a fraction containing a large amount of cell membranes obtained by a per se known method after disruption of the cells. Examples of methods of cell disruption include crushing in a Potter-Elvehjem homogenizer, disruption in a Waring blender or Polytron (Kinematica), disruption by ultrasonication, disruption by squirting through a narrow nozzle while applying pressure with a French press etc., and the like. For fractionation of the cell membranes, methods employing centrifugal force such as fractionation centrifugation, density-gradient centrifugation, and the like are mainly used. For example, a suspension of disrupted cells is centrifuged at low speed (500 to 3000 rpm) for a short period of time (normally, about 1 to 10 minutes); the supernatant is then further centrifuged at high speed (15,000 to 30,000 rpm), normally for 30 minutes to 2 hours, and the sediment obtained is regarded as membrane fraction. This cell membrane fraction contains the expressed receptor protein and many membrane components, such as phospholipids, membrane proteins, and the like.

The amount of receptor protein in cells containing the receptor protein, and in the membrane fraction from such cells, is preferably 10^3 to 10^8 molecules per cell, and more preferably 10^5 to 10^7 molecules per cell. At higher levels of expression, ligand binding activity (specific activity) of the membrane fraction is increased, which makes possible not only the construction of a highly sensitive screening system, but also determination of a large number of samples in one lot.

To carry out methods ① to ③ above of screening for compounds that alter the binding of ligands to the receptor protein of the present invention, for example, a suitable receptor fraction and labelled ligand are necessary.

The receptor protein fraction is preferably a naturally occurring receptor protein fraction, a recombinant receptor protein fraction having activity equivalent to that, or the like. Used herein, the term "equivalent activity" means equivalent ligand binding activity, signal information transducing activity, and the like.

A labelled ligand, labelled ligand analog compound, or the like can be used as the labelled ligand. For example, ligands labelled with [^{3}H], [^{125}I], [^{14}C], [^{35}S], or the like can be used.

Specifically, to carry out screening for compounds that alter the binding of ligands to the receptor protein of the present invention, first a receptor protein sample is prepared by suspending cells containing the receptor protein of the present invention, or the membrane fraction such cells, in a buffer appropriate for screening. Any buffer that does not interfere with binding of the ligand to the receptor protein can be used, for example, a phosphate buffer, Tris-HCl buffer, or the like of pH 4 to 10 (preferably pH 6 to 8). To decrease nonspecific binding, a surfactant such as CHAPS, Tween 80 ™ (Kao-Atlas), digitonin, deoxycholate, or the like can be added to the buffer. In addition, to inhibit degradation of the receptor protein and ligand by proteases, a protease inhibitor such as PMSF, leupeptin, E-64 (Peptide Institute), pepstatin, or the like can also be added to the buffer. A given amount (5000 to 500,000 cpm) of labelled ligand is added to 0.01 to 10 ml of receptor suspension, and at the same time, test compound at a concentration of 10^{-4} M to 10^{-10} M is also allowed to coexist. To determine the amount of nonspecific binding, a reaction tube with a large excess of unlabelled ligand is also prepared. The reaction is carried out at about 0°C to 50°C, preferably about 4°C to 37°C, for about 20 minutes to 24 hours, preferably about 30 minutes to 3 hours. After the reaction, [the reaction mixture] is filtered through a glass fiber filter; then, after [the filter] has been washed with a suitable amount of the same buffer, the radioactivity remaining on the filter is measured using a liquid scintillation counter or γ-counter. When the count (B_0 – NSB) obtained by subtracting nonspecific binding (NSB) from the count when there is no antagonistic substance (B_0) is considered to be 100%, test compounds that result in specific binding (B – NSB) of, for example, 50% or less can be selected as candidates for substances capable of inhibiting antagonism.

To carry out methods ④ and ⑤ above of screening for compounds that alter the binding of ligands to the receptor protein of the present invention, for example, receptor protein-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) can be measured by a known method or by a commercially available kit.

Specifically, the cells containing the receptor protein of the present invention are first cultured in a multiwell plate or the like. At the time of screening, before addition of the test

compound, [the culture medium] is replaced with fresh medium or with an appropriate buffer that is not toxic to the cells. Then test compound etc. is added and the culture is incubated for a given period of time. After the incubation, the cells are extracted or the supernatant is recovered, and the products formed are quantified by suitable methods. When assay of the formation of the substance (e.g., arachidonic acid, etc.) serving as an index of cell stimulation activity is difficult because of a degradative enzyme in the cells, the assay can be performed after addition of an inhibitor of the degradative enzyme. In addition, cAMP production-inhibiting activity and the like can be detected as inhibition of production in cells whose basal production has been increased by forskolin, etc.

To carry out screening by measuring cell stimulation activity, cells expressing a suitable receptor protein are necessary. Preferred cells expressing the receptor protein of the present invention include naturally occurring cell strains expressing the receptor protein of the present invention, cells strains expressing recombinant receptor protein described above, or the like.

Examples of test compounds that can be used include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, and the like. These compounds may be novel or known.

Kits for screening for compounds or salts thereof that alter the binding of ligands to the receptor protein of the present invention contain the receptor protein of the present invention, cells containing the receptor protein of the present invention, the membrane fraction of cells containing the receptor protein of the present invention, or the like.

The following are examples of a kit for screening of the present invention.

1. Reagents for Screening

① Measurement buffer and wash buffer

Hanks' balanced salt solution (Gibco) with 0.05% bovine serum albumin (Sigma) added to it.

The buffer is either sterilized by filtration through a filter with a pore diameter of 0.45 μm and then stored at 4°C, or prepared at the time of use.

② G protein-coupled receptor sample

CHO cells expressing the receptor protein of the present invention passaged into 12-well plates at 5 x 10^5 cells/well, and cultured at 37°C, in 5% CO_2 and 95% air, for 2 days.

③ Labelled ligand

A ligand labelled with commercially available [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc.

[Labelled ligand] is stored in the form of an aqueous solution at 4°C or -20°C; at the time of use, the solution is diluted to 1 μM with measurement buffer.

④ Ligand standard solution

Ligand is dissolved to a final concentration of 1 mM in PBS containing 0.1% bovine serum albumin (Sigma), and [the solution is] stored at -20°C.

2. Measurement Method

① CHO cells expressing the receptor protein of the present invention cultivated in a 12-well tissue culture plate are washed twice with 1 ml of measurement buffer. Then 490 µl of measurement buffer is added to each well.

② Next, 5 µl of 10^{-3}- to 10^{-10}-M test compound solution is added. Then 5 µl of labelled ligand is added, and the reaction is allowed to proceed at room temperature for 1 hour. To determine the amount nonspecific binding, 5 µl of 10^{-3}-M ligand is added in place of the test compound solution.

③ The reaction mixture is removed, and washing with 1 ml of wash buffer is done 3 times. Labelled ligand bound to the cells is dissolved with 0.2-N NaOH-1% SDS and mixed with 4 ml of liquid scintillator A (Wako Pure Chemicals Industries).

④ Radioactivity is measured with a liquid scintillation counter (Beckman), and percent maximum binding is determined according to the following formula (Formula 1).

Formula 1

$$PMB = [(B - NSB)/(B_0 - NSB)] \times 100$$

PMB : Percent maximum binding
B : [Binding] value obtained when a specimen is added
NSB : Nonspecific binding
B_0 : Maximum binding

Compounds and salts thereof obtained using the screening method or screening kit of the present invention are compounds that have the action of altering the binding of ligands to the receptor protein of the present invention; specifically, (a) compounds that have G protein-coupled receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) (so-called "agonists" of the receptor protein of the present invention), (b) compounds that do not have said cell stimulation activity (so-called "antagonists" of the receptor protein of the present invention), (c) compounds that increase the binding strength between ligands and the G protein-coupled receptor protein of the present invention, and (d) compounds that decrease the binding strength between ligands and the G protein-coupled receptor protein of the present invention.

Examples of the compounds include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, and the like. These compounds may be novel or known.

Because agonists of the receptor protein of the present invention have action which is the same as the physiological activity of ligands for the receptor protein, the agonists are useful as safe, low-toxicity drugs providing the ligand activity.

Because antagonists of the receptor protein of the present invention can inhibit the physiological activity of ligands for the receptor protein, the antagonists are useful as safe, low-toxicity drugs for inhibiting ligand activity.

Compounds that increase the binding strength between ligands and the G protein-coupled receptor protein of the present invention are useful as safe, low-toxicity drugs for increasing the physiological activity of ligands for the receptor protein of the present invention (for example, drugs for preventing and/or treating central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.]).

Compounds that decrease the binding strength between ligands and the G protein-coupled receptor protein of the present invention are useful as safe, low-toxicity drugs for decreasing the physiological activity of ligands for the receptor protein of the present invention.

When a compound or salt thereof obtained using the screening method or screening kit of the present invention is used as an above-described pharmaceutical composition, [the compostion] can be used by conventional means. For example, in the same way as drugs containing the receptor protein of the present invention described above, [the composition] may be a tablet, capsule, elixir, microcapsule, sterile solution, suspension, or the like.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(8) Drugs for Preventing and/or Treating Various Diseases Containing Compounds (Agonists, Antagonists) That Alter the Binding of Ligands to the G Protein-coupled Receptor Protein of the Present Invention

As explained above, the receptor protein of the present invention is thought to play some important role in the body, for example a central function. Therefore, compounds (agonists,

antagonists) that alter the binding of ligands to the receptor protein can be used as drugs for preventing and/or treating diseases related to insufficient function of the receptor protein.

When such a compound is used as a drug for preventing and/or treating diseases related to insufficient function of the receptor protein of the present invention (for example, central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.] etc.), [the compound] can be formulated by conventional means.

For example, the compound may be used orally in the form of tablets that may be sugar coated as necessary, capsules, elixirs, microcapsules, and the like, or parenterally in the form of injectable preparations such as sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. These preparations can be produced, for example, by mixing the compound with physiologically acceptable known carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, binders, and the like in unit dosage forms required for generally accepted pharmaceutical practice. The amount of active ingredient in these preparations is set so that an appropriate dose within the specified range is obtained.

Additives that can be incorporated in tablets, capsules, and the like include, for example, binders such as gelatin, corn starch, tragacanth, and gum arabic; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose, and saccharin; and flavoring agents such as peppermint, akamono oil, and cherry. When the unit dosage form is a capsule, the above-mentioned materials may also include liquid carriers such as oils and fats. Sterile compositions for injection can be formulated according to ordinary pharmaceutical practice such as by dissolving or suspending the active ingredient and naturally occurring vegetable oils such as sesame oil, coconut oil, and the like in a vehicle such as water for injection. Aqueous liquids for injection that may be used include, for example, physiological saline and isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride), and the aqueous liquids may be used in combination with appropriate solubilizing agents such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80 ™, HCO-50), and the like. Oily liquids that may be used include, for example, sesame oil and soybean oil, and the oily liquids may be used in combination with solubilizing agents such as benzyl benzoate and benzyl alcohol.

Furthermore, the preventive or therapeutic drugs described above may also be combined with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene

glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, and the like. The prepared solution for injection is normally packaged in an appropriate ampule.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(9) Quantifying the Receptor Protein of the Present Invention, Peptide Fragments Thereof, and Salts Thereof

Because the antibody of the present invention can specifically recognize the receptor protein of the invention, the antibody can be used for quantification of the receptor protein in a liquid test sample, and in particular, for quantification by sandwich immunoassay, and so on. That is, the present invention provides, for example:

(i) A method for quantifying the receptor protein of the present invention in a liquid test sample, the method being characterized by competitively reacting the antibody of the present invention with the liquid test sample and labelled receptor protein and measuring the percentage of labelled protein that binds to the antibody; and

(ii) A method for quantifying the receptor protein of the present invention in a liquid test sample, the method being characterized by reacting the liquid test sample with the antibody of the present invention insolubilized on a carrier and with labelled antibody of the present invention, either simultaneously or consecutively, and subsequently measuring the activity of the labeling agent on the insolubilized carrier.

In (ii) above, it is preferable that one antibody be an antibody that recognizes the N-terminal region of the receptor protein of the present invention, and the other antibody be an antibody that reacts with the C-terminal region of the receptor protein.

In addition to allowing measurement of the receptor protein of the present invention, the usage of a monoclonal antibody to the receptor protein of the invention (hereinafter sometimes referred to as the "monoclonal antibody of the present invention") also allows detection by histological staining and so on. For these purposes, the antibody molecule itself, or the $F(ab')_2$,

Fab', or Fab fraction of the molecule may be used. A measurement method using an antibody to the receptor protein of the present invention does not have to be specifically limited; any measurement method can be used provided that it is a method in which an amount of antibody, antigen, or antigen-antibody complex corresponding to the amount of antigen (e.g., amount of receptor protein) in the liquid assay sample is detected by chemical or physical means, and calculated from a standard curve prepared using standard solutions containing known amounts of antigen. For example, nephelometry, competitive methods, immunometric methods, and sandwich methods are suitably employed, with use of the sandwich method described hereinafter being especially preferable in view of sensitivity and specificity.

Labelling agents that may be used in measurement methods that employ labelled substances include, for example, radioisotopes, enzymes, fluorescent materials, luminescent materials, and the like. Radioisotopes that may be used include, for example, [^{125}I], [^{131}I], [^{3}H], [^{14}C], and the like. As enzymes that may be used, stable ones with high specific activity are preferable, for example, β- galactosidase, β-glucosidase, alkaline phosphatase, peroxidase, malate dehydrogenase, and the like. Fluorescent materials that may be used include, for example, fluorescamine, fluorescein isothiocyanate, and the like. Luminescent materials that may be used include, for example, luminol, luminol derivatives, luciferin, lucigenin, and the like. In addition, a biotin-avidin system can be used for binding an antibody or an antigen to a labelling agent.

For insolubilization of antigen or antibody, physical adsorption can be used, or methods employing chemical bonding that is normally used to insolubilize and immobilize proteins, enzymes, or the like can be used. Carriers that may be used include, for example, insoluble polysaccharides such as agarose, dextran, cellulose and the like; synthetic resins such as polystyrene, polyacrylamide, silicone, and the like; and glass and the like.

In the sandwich method, the amount of receptor protein of the present invention in a liquid test sample can be measured by reacting the liquid test sample with insolubilized monoclonal antibody of the present invention (primary reaction), reacting labelled monoclonal antibody of the present invention (secondary reaction), and then measuring the activity of the labeling agent on the insolubilized carrier. The primary and secondary reactions can be carried out in the reverse order; in addition, they can be carried simultaneously, or separately at different times. The labelling agent and insolubilization method may be as those described above.

In addition, in immunoassays by a sandwich method, the antibody used as the solid-phase antibody or the labelled antibody does not necessarily have to be one kind; a mixture of 2 or more antibodies may be used to improve measurement sensitivity etc.

In the method for measuring the receptor protein by a sandwich method of the present invention, it is preferable to use antibodies that bind to different sites on the receptor protein as the monoclonal antibodies of the present invention employed in the primary and secondary reactions. For example, when the antibody used in the secondary reaction recognizes the C-terminal region of the receptor protein, it is preferable to use an antibody recognizing a region other than the C-terminal region, for example the N-terminal region, in the primary reaction.

The monoclonal antibodies of the present invention can also be used in measurement systems other than the sandwich method, for example, in competitive methods, immunometric methods, nephelometry, and the like. In the competitive methods, the amount of antigen in a liquid test sample is measured by reacting an antibody competitively with labelled antigen and the antigen in the test sample, then separating unreacted [*sic*] labelled antigen (F) from antibody-bound labelled antigen (B) (B/F separation), and measuring the amount of label in either F or B. In the reaction, a liquid phase method or a solid phase method can be used. In the liquid phase method, a soluble antibody is used as the antibody, and B/F separation is performed using polyethylene glycol, a second antibody to the above-mentioned antibody, and so on. In the solid phase method, an immobilized solid phase antibody is used as the first antibody, or a soluble antibody is used as the first antibody and an immobilized solid phase antibody is used as the second antibody.

In the immunometric methods, antigen in a liquid test sample and immobilized solid phase antigen are competitively reacted with a given amount of a labelled antibody, and then the solid phase is separated from the liquid phase. Alternatively, antigen in a liquid test sample is reacted with an excess of labelled antibody; next, immobilized solid phase antigen is added so that unreacted labelled antibody can bind to it; and then the solid phase is separated from the liquid phase. Finally, the amount of antigen in the liquid test sample is determined by measuring the amount of label in either phase.

In nephelometry, an antigen-antibody reaction is carried out in a gel or solution, and the amount of insoluble precipitate formed is measured. Even when the amount of antigen in a liquid test sample is small and only a small amount of precipitate is obtained, laser nephelometry, which uses laser scattering, and the like can be suitably employed.

When employing these immunoassay methods as measurement methods of the present invention, it is not necessary to establish any special conditions, operations, or the like. A measurement system for the receptor protein or a salt thereof of the present invention may be constructed based on the ordinary conditions and operating procedures of each method together with the ordinary technical consideration of a person skilled in the art. As for details of these general technical means, reference can be made to various reviews, texts and the like, for example, Hiroshi Irie (ed), *Rajioimunoassei* [translation: Radioimmunoassay] (Kodansha, 1974); Hiroshi Irie (ed), *Zoku Rajioimunoassei* [translation: Radioimmunoassay Continued] (Kodansha, 1979); Eizi Ishikawa et al. (ed.), *Koso Meneki Sokuteiho* [translation: Enzyme Immunoassay], Igaku-shoin (1978); Eizi Ishikawa et al. (ed.), *Koso Meneki Sokuteiho Dai 2 Ban* [translation: Enzyme Immunoassay, 2nd edition], Igaku-shoin (1982); Eizi Ishikawa et al., (ed.), *Koso Meneki Sokuteiho Dai 3 Ban* [translation: Enzyme Immunoassay, 3rd edition], Igaku-shoin (1987); *Methods in Enzymology*, Vol. 70, "Immunochemical Techniques (Part A)"; *ibid.*, Vol. 73, "Immunochemical Techniques (Part B)"; *ibid.*, Vol. 74, "Immunochemical Techniques (Part C)"; ibid., Vol. 84, "Immunochemical Techniques (Part D: Selected Immunoassays)"; *ibid.*, Vol. 92, "Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay

Methods)"; *ibid.*, Vol. 121, "Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)" (the above [*Methods in Immunology* volumes] are published by Academic Press); and the like.

As described above, the receptor protein or a salt thereof of the present invention can be quantified with high sensitivity using antibodies of the present invention.

Furthermore, by quantifying the receptor protein or salts thereof of the present invention in the body using antibodies of the present invention, various diseases related to insufficient function of the receptor protein (for example, central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.] etc.) can be diagnosed.

In addition, the antibodies of the present invention can be used to specifically detect the receptor protein of the present invention in samples of body fluid, tissue, and the like. The antibodies of the present invention can also be used to prepare antibody columns for purification of the receptor protein of the present invention, to detect the receptor protein in the fractions at the time of purification, to analyze the behavior of the receptor protein in cells under study, and so on.

(10) Methods of Screening for Compounds That Alter the Amount of the Receptor Protein or Peptide Fragments Thereof of the Present Invention in the Cell Membrane

Because the antibodies of the present invention can specifically recognize the receptor protein, peptide fragments thereof, or salts thereof of the present invention, the antibodies can be used to screen for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane.

That is, the present invention provides, for example:

(i) A method of screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane, the screening method being to disrupt ① blood, ② specific organs, or ③ tissues or cells isolated from organs, etc., of nonhuman mammals, isolate the cell membrane fraction, and quantify the receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction;

(ii) A method of screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane, the screening method being to disrupt transformants etc. expressing the receptor protein or peptide fragments thereof of the present invention, isolate the cell membrane fraction, and quantify the receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction;

(iii) A method of screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane, the screening method being to section ① blood, ② specific organs, or ③ tissues or cells isolated from organs, etc., of nonhuman mammals, and then using an immunostaining method, quantify the extent of staining of the receptor protein in the cell surface layer, and thereby confirm the protein on the cell membrane;

(iv) A method of screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane, the screening method being to section a transformant etc. expressing the receptor protein or peptide fragments thereof of the present invention, and then using an immunostaining method, quantify the extent of staining of the receptor protein in the cell surface layer, and thereby confirm the protein on the cell membrane.

Specifically, quantification of the receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction is performed as described below.

(i) Normal or disease model nonhuman mammals (e.g., mice, rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.; more specifically demented rats, obese mice, arteriosclerotic rabbits, tumor-bearing mice, etc.) are subjected to drugs (e.g., antidementia agents, hypotensive agents, antineoplastic agents, antiobesity agents, etc.), physical stress (e.g., water immersion stress, electric shock, light/darkness, low temperature, etc.), or the like, and after a fixed period of time, blood, specific organs (e.g., brain, liver, kidney, etc.), or tissue or cells isolated from organs, are obtained. The organs, tissues, cells, etc. obtained, are, for example, suspended in a suitable buffer (e.g., Tris-HCl buffer, phosphate buffer, HEPES buffer, etc.) etc. and disrupted. Then, using a surfactant (e.g., Triton X-100 ™, Tween 20 ™, etc.) etc., the disrupted organs, tissues, or cells are centrifuged and filtered, and the cell membrane fraction is obtained by a technique such as column fractionation.

A cell membrane fraction is a fraction containing a large amount of cell membranes obtained by a per se known method after disruption of the cells. Examples of methods of cell disruption include crushing in a Potter-Elvehjem homogenizer, disruption in a Waring blender or Polytron (Kinematica), disruption by ultrasonication, disruption by squirting through a narrow nozzle while applying pressure with a French press etc., and the like. For fractionation of the cell membranes, methods employing centrifugal force such as fractionation centrifugation, density-gradient centrifugation, and the like are mainly used. For example, a suspension of disrupted cells is centrifuged at low speed (500 to 3000 rpm) for a short period of time (normally, about 1 to 10 minutes); the supernatant is then further centrifuged at high speed (15,000 to 30,000 rpm), normally for 30 minutes to 2 hours, and the sediment obtained is regarded as membrane fraction. This cell membrane fraction contains the expressed receptor protein and many membrane components, such as phospholipids, membrane proteins, and the like.

The receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction can, for example, be quantified by sandwich immunoassay, Western blotting, and the like using antibodies of the present invention.

The sandwich immunoassay can be performed the same as the method described above, and Western blotting according to per se known means.

(ii) A transformant expressing the receptor protein or a peptide fragment thereof of the present invention can be prepared according to the method described above, and the receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction can be quantified.

Screening for compounds that alter the amount of the receptor protein or peptide fragments thereof of the present invention in the cell membrane can be performed by,

(i) Administering a test compound to normal or disease model nonhuman mammals at a fixed period of time before (30 minutes to 24 hours before, preferably 30 minutes to 12 hours before, more preferably 1 hour to 6 hours before), a fixed period of time after (30 minutes to 3 days after, preferably 1 hour to 2 days after, more preferably 1 hour to 24 hours after), or at the same time as the animals are subjected to drugs or physical stress, and quantifying the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane at a fixed period of time after (30 minutes to 3 days after, preferably 1 hour to 2 days after, more preferably 1 hour to 24 hours after) administration; and

(ii) Adding a test compound to the culture medium when culturing a transformant by an ordinary method; then after (1 to 7 days after, preferably 1 to 3 days after, more preferably 2 to 3 days after) culture for a fixed period of time, quantifying the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane.

Specifically, confirmation of receptor protein or peptide fragments thereof of the present invention in the cell membrane fraction is performed as described below.

(iii) Normal or disease model nonhuman mammals (e.g., mice, rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.; more specifically demented rats, obese mice, arteriosclerotic rabbits, tumor-bearing mice, etc.) are subjected to drugs (e.g., antidementia agents, hypotensive agents, antineoplastic agents, antiobesity agents, etc.), physical stress (e.g., water immersion stress, electric shock, light/darkness, low temperature, etc.), or the like, and after a fixed period of time, blood, specific organs (e.g., brain, liver, kidney, etc.), or tissue or cells isolated from organs, are obtained. Sections are prepared by a conventional method from the organs, tissues, cells, etc. that are obtained, and immunostaining is performed using antibodies of the present invention. Then by confirming the receptor protein on the cell membrane by quantifying the extent of staining of the receptor protein in the cell surface layer, the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane can be confirmed quantitatively or qualitatively.

(iv) Confirmation can also be accomplished by the same means using a transformant or the like expressing the receptor protein or peptide fragments thereof of the present invention.

Compounds or salts thereof obtained by using the screening method of the present invention are compounds that have the action of altering the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane; specifically, (a) compounds that increase G protein-coupled receptor-mediated cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) by increasing the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane; and (b) compounds that decrease the cell stimulation activity by decreasing the amount of receptor protein or peptide fragments thereof of the present invention in the cell membrane.

Examples of the compounds include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, and the like. These compounds may be novel or known.

Compounds that increase the cell stimulation activity are useful as safe drugs of low toxicity (for example, drugs for preventing and/or treating central diseases [e.g., Alzheimer disease, dementia, eating disorders {cibophobia}, epilepsy, etc.], hormone system diseases [e.g., weak labor pain, atonic hemorrhage, status peri-afterbirth, insufficient uterine involution, cesarean section, induced abortion, galactostasis, etc.], hepatic/biliary/pancreatic/endocrine diseases [e.g., diabetes, eating disorders, etc.], inflammatory diseases [e.g., allergy, asthma, rheumatism, etc.), and cardiovascular diseases [e.g., hypertension, cardiomegaly, angina pectoris, arteriosclerosis, etc.]) that increase the physiological activity of the receptor protein of the present invention.

Compounds that decrease the cell stimulation activity are useful as safe drugs of low toxicity that decrease the physiological activity of the receptor protein of the present invention.

When a compound or salt thereof obtained using the screening method of the present invention is used as a pharmaceutical composition, [the compostion] can be practiced by conventional means. For example, in the same way as drugs containing the receptor protein of the present invention described above, [the composition] may be a tablet, capsule, elixir, microcapsule, sterile solution, suspension, or the like.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more

preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(11) Drugs for Preventing or Treating Various Diseases Containing Compounds That Alter the Amount of the Receptor Protein or Peptide Fragments Thereof of the Present Invention in the Cell Membrane

As explained above, the receptor protein of the present invention is thought to play some important role in the body, for example a central function. Therefore, compounds that alter the amount of the receptor protein or peptide fragments thereof of the invention in the cell membrane can be used as drugs for preventing and/or treating diseases related to insufficient function of the receptor protein.

When such a compound is used as a drug for preventing and/or treating diseases related to insufficient function of the receptor protein of the present invention, [the compound] can be formulated by conventional means.

For example, the compound may be used orally in the form of tablets that may be sugar coated as necessary, capsules, elixirs, microcapsules, and the like, or parenterally in the form of injectable preparations such as sterile solutions or suspensions prepared with water or other pharmaceutically acceptable liquids. These preparations can be produced, for example, by mixing the compound with physiologically acceptable known carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, binders, and the like in unit dosage forms required for generally accepted pharmaceutical practice. The amount of active ingredient in these preparations is set so that an appropriate dose within the specified range is obtained.

Additives that can be incorporated in tablets, capsules, and the like include, for example, binders such as gelatin, corn starch, tragacanth, and gum arabic; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose, and saccharin; and flavoring agents such as peppermint, akamono oil, and cherry. When the unit dosage form is a capsule, the above-mentioned materials may also include liquid carriers such as oils and fats. Sterile compositions for injection can be formulated according to ordinary pharmaceutical practice such as by dissolving or suspending the active ingredient and naturally occurring vegetable oils such as sesame oil, coconut oil, and the like in a vehicle such as water for injection. Aqueous liquids for injection that may be used include, for example, physiological saline and isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride), and the aqueous liquids may be used in combination with appropriate solubilizing agents such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80 ™, HCO-50), and the like. Oily liquids that may be used include, for example, sesame oil and soybean oil, and the oily liquids may be used in combination with solubilizing agents such as benzyl benzoate and benzyl alcohol.

Furthermore, the preventive or therapeutic drugs described above may also be combined with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene

glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, and the like. The prepared solution for injection is normally packaged in an appropriate ampule.

Because the preparations obtained in the above manner are safe and low in toxicity, they can be administered to humans and [other] mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.).

Although there are differences depending on the subject of administration, target organ, symptoms, administration method, and the like, generally the daily dose of the compound or salt thereof for oral administration is, in cibophobia patients (weighing 60 kg) for example, about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg. For parenteral administration, although the size of a single dose varies depending on the subject of administration, target organ, symptoms, administration method, and the like, ordinarily, for an injectable preparation, it is favorable to administer, in cibophobia patients (weighing 60 kg) for example, a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg by intravenous injection. In other animals, corresponding doses converted per 60 kg can be administered.

(12) Neutralization by Antibodies to the Receptor Protein, Peptide Fragments Thereof, or Salts Thereof of the Present Invention

The neutralizing activity of antibodies to the receptor protein, peptide fragments thereof, or salts thereof of the present invention on those substances refers to activity inactivating signal transduction functions in which the receptor protein is involved. When the antibodies have neutralizing activity, they can inactivate signal transduction in which the receptor protein is involved, for example, cell stimulation activity (for example, activity enhancing or inhibiting such things as arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential changes, intracellular protein phosphorylation, c-fos activation, pH decrease, etc.) mediated by the receptor protein. Therefore, antibodies with neutralizing activity can be used for preventing and/or treating diseases caused by overexpression of the receptor protein and the like.

(13) Creation of Nonhuman Animals Having DNA Encoding the G Protein-coupled Receptor Protein of the Present Invention

Using the DNA of the present invention, nonhuman transgenic animals that express the receptor protein of the present invention can be generated. As the nonhuman animals, mammals (e.g., rats, mice, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, etc.) and the like (hereinafter referred to as "animals") can be mentioned, with mice, rabbits, and the like being especially suitable.

In transferring the DNA of the present invention to a host animal, it is generally advantageous to use the DNA as a gene construct in which the DNA is conjugated downstream

of a promoter capable of causing expression in animal cells. For example, when rabbit-derived DNA of the present invention is transferred, DNA transgenic animals for high production of the receptor protein of the present invention can be generated by the microinjection of, for example, the fertilized rabbit egg with gene constructs in which animal-derived DNA of the present invention having high homology to rabbit-derived DNA of the present invention is conjugated downstream of various promoters capable of causing expression in animal cells. As for such promoters, for example, virus-derived promoters and ubiquitous expression promoters such as the metallothionein promoter can be used, but promoters that are specifically expressed in the brain such as the NGF gene promoter and the enolase gene promoter are preferable.

Transfer of the DNA of the present invention at the fertilized-egg-cell stage ensures that the DNA will be present in all the germ cells and somatic cells of the target animal. Presence of the receptor protein of the present invention in the germ cells of the animal generated following DNA transfer means that the receptor protein is present in all germ cells and somatic cells of all the progeny of the animal. Progeny of animals of the species that inherit the gene have the receptor protein of the present invention in all of their germ cells and somatic cells.

It can be confirmed by mating that the DNA-transferred animal of the present invention stably retains the gene, and the animal can be bred as DNA-harboring animals from generation to generation in an ordinary environment for animal husbandry. Furthermore, by mating male and female animals harboring the objective DNA, it is possible to obtain homozygous animals having the introduced gene in both homologous chromosomes, and by mating such homozygous males and females, it is possible to breed the animals ensuring that all progeny harbor the DNA.

Because the receptor protein of the present invention is highly expressed in animals into which the DNA of the present invention has been transferred, these animals have uses including screening for agonists and antagonists of the receptor protein of the present invention.

DNA-transferred animals of the present invention can also be used as a source of cells for tissue culture. For example, the receptor protein of the present invention can be studied either by directly analyzing DNA or RNA in the tissues of a mouse into which the DNA of the present invention was transferred, or by analyzing tissues containing the receptor protein of the present invention expressed by the gene. It is possible to culture cells of tissues containing the receptor protein of the present invention by standard tissue culture techniques, and using those cells, for example, study the functions of cells from tissues that are generally difficult to culture, such as cells of the brain or peripheral tissue. Furthermore, by using such cells, it is possible, for example, to select drugs that increase functions of various tissues. Moreover, if there is a cell line with high expression, it will be possible to isolate and purify the receptor protein of the present invention from cells of that line.

In the present specification and drawings, the abbreviations used for bases, amino acids, and so forth are based on those of the IUPAC-IUB Commission on Biochemical Nomenclature and those conventionally used in the art. Examples thereof are given below. Furthermore, amino

acids for which optical isomerism is possible are in the L form unless otherwise specified.

DNA : Deoxyribonucleic acid
cDNA : Complementary deoxyribonucleic acid
A : Adenine
T : Thymine
G : Guanine
C : Cytosine
RNA : Ribonucleic acid
mRNA : Messenger ribonucleic acid
dATP : Deoxyadenosine triphosphate
dTTP : Deoxythymidine triphosphate
dGTP : Deoxyguanosine triphosphate
dCTP : Deoxycytidine triphosphate
ATP : Adenosine triphosphate
EDTA : Ethylenediaminetetraacetic acid
SDS : Sodium dodecyl sulfate
Gly : Glycine
Ala : Alanine
Val : Valine
Leu : Leucine
Ile : Isoleucine
Ser : Serine
Thr : Threonine
Cys : Cysteine
Met : Methionine
Glu : Glutamic acid
Asp : Aspartic acid
Lys : Lysine
Arg : Arginine
His : Histidine
Phe : Phenylalanine
Tyr : Tyrosine
Trp : Tryptophan
Pro : Proline

Asn : Asparagine
Gln : Glutamine
pGlu : Pyroglutamic acid
Me : Methyl group
Et : Ethyl group
Bu : Butyl group
Ph : Phenyl group
TC : Thiazolidine-4(R)-carboxamide

In addition, substitution groups, protective groups, and reagents frequently used in the present specification are represented by the symbols set forth below.

Tos : p-Toluene sulfonyl
CHO : Formyl
Bzl : Benzyl
Cl_2Bzl : 2,6-Dichlorobenzyl
Bom : Benzyloxymethyl
Z : Benzyloxycarbonyl
Cl-Z : 2-Chlorobenzyloxycarbonyl
Br-Z : 2-Bromobenzyloxycarbonyl
Boc : tert-Butoxycarbonyl
DNP : Dinitrophenol
Trt : Trityl
Bum : tert-Butoxymethyl
Fmoc : N-9- Fluorenylmethoxycarbonyl
HOBt : 1-Hydroxybenzotriazole
HOOBt : 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
HONB : 1-Hydroxy-5-norbornene-2,3-dicarboximide
DCC : N,N'-Dicyclohexylcarbodiimide

Each SEQ ID NO set forth in the SEQUENCE LISTING of the specification refers to the following sequence:

SEQ ID NO:1
The amino acid sequence of novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention.

SEQ ID NO:2
The base sequence of the cDNA encoding the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention having the amino acid sequence of SEQ ID NO:1.

SEQ ID NO:3
The base sequence of Primer-1 used to clone the cDNA encoding the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention.

SEQ ID NO:4
The base sequence of Primer-2 used to clone the cDNA encoding the novel G protein-coupled receptor protein hSLT of human hippocampal origin of the present invention.

Escherichia coli DH5α/pCR3.1-hSLT obtained in Example 1 below has been on deposit since 28 April 1999 under Accession Number FERM BP-6710 at the National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science and Technology, Ministry of International Trade and Industry, 1-1-3 Higashi, Tsukuba-shi, Ibaraki-ken, Japan; and since 20 April 1999 under Accession Number IFO 16284 at the Institute for Fermentation (IFO), 2-17-85 Juso-honmachi, Yodogawa-ku, Osaka-shi, Osaka-fu, Japan.

Examples

Below, reference examples [sic] and working examples are shown. The examples explain the invention in more detail, but they do not limit its scope. The gene manipulations using *Escherichia coli* were performed according to *Molecular Cloning*.

Working Example 1: Cloning of a cDNA encoding the human hippocampal G protein-coupled receptor protein, and determination of the base sequence

PCR was carried out on a human hippocampal cDNA (CLONTECH) template using 2 primers, Primer-1 (SEQ ID NO:3) and Primer-2 (SEQ ID NO:4). As for the composition of the reaction mix, 1/10 volume of the above cDNA was used as the template, and to that were added 1/50 volume of Advantage 2 Polymerase Mix (CLONTECH), Primer-1 (SEQ ID NO:3) and Primer-2 (SEQ ID NO:4), each 0.2 μM, dNTPs 200 μM, and buffer with enzyme to bring the final volume to 25 μl. PCR was carried out under conditions of ① 94 °C for 1 min, then, ② 3 cycles of 94 °C for 20 s and 72 °C for 2 min, ③ 3 cycles of 94 °C for 20 s, 65 °C for 20 s, and 68 °C for 2 min, and ④ 36 cycles of 94 °C for 20 s, 58 °C for 20 s, and 68 °C for 2 min, and ⑤ finally an elongation reaction at 68 °C for 7 min. After the PCR, the reaction products were subcloned into plasmid vector pCR3.1 (Invitrogen) according to the method of the TA Cloning Kit (Invitrogen). The plasmids were then introduced into *Escherichia coli* DH5α, and clones containing cDNA were selected with LB agar medium containing ampicillin. The sequence of each clone was then analyzed, and as a result, a cDNA sequence (SEQ ID NO:2) encoding a novel G protein-coupled receptor protein was obtained. The novel G protein-coupled receptor protein containing the amino acid sequence deduced from the cDNA (SEQ ID NO:1) was named "hSLT", and the transformant was named "*Escherichia coli* DH5α/pCR3.1-hSLT".

Working Example 2: Preparation of CHO cells expressing hSLT

E. coli DH5α (Toyobo) transformed with plasmid pCR3.1-hSLT, into which the gene encoding the full-length amino acid sequence of hSLT of human hippocampal origin whose sequence was determined in Working Example 1 had been inserted, was cultured, and pCR3.1-hSLT plasmid DNA was prepared using a Plasmid Midi Kit (Qiagen). Plasmid DNA was then introduced into CHO-K1 cells using a CellPhect Transfection Kit (Amersham Pharmacia Biotech) according to the protocol provided with the kit. A suspension of 10 mg of plasmid DNA coprecipitated with calcium phosphate was added to a 10-cm dish onto which 5×10^5 or 1×10^6 CHO-K1 cells had been plated 24 hours earlier. After culture in DMEM medium containing 10% fetal calf serum for one day, the cells were passaged and cultured in the selection medium, which was DMEM containing 10% fetal calf serum and 1 mg/ml G418. From the hSLT-expressing CHO cell colonies that grew in the selection medium, 40 clones were selected. Furthermore, total RNA was extracted by the usual method from the selected hSLT-expressing CHO cells, the amount of hSLT mRNA was quantified by Taq Man PCR, and copy

number was determined. As a result, 3 clones (#5, #7, and #28) were selected as lines with high expression.

Industrial Applicability

The G protein-coupled receptor protein of the present invention, peptide fragments thereof, or salts thereof, and polynucleotides (e.g., DNA, RNA, and their derivatives) encoding the receptor protein, peptide fragment thereof, and salts thereof can be used (1) to determine ligands (agonists), (2) to obtain antibodies or antisera, (3) to construct expression systems for recombinant receptor protein, (4) to develop receptor binding assay systems using the expression systems and screen for drug candidate compounds, (5) to carry out drug design based on comparison with structurally similar ligands and receptors, (6) to serve as reagents for the construction of probes and PCR primers for genetic diagnosis, (7) to prepare transgenic animals, and (8) to serve as drugs such as a genetic prevention/treatment drug, and so on.

Claims

1. A protein, or a salt thereof, which is characterized by comprising an amino acid sequence the same as, or substantially the same as, the amino acid sequence of SEQ ID NO:1.
2. A peptide fragment, or a salt thereof, of the protein of claim 1.
3. A polynucleotide comprising a polynucleotide which has a base sequence encoding the protein of claim 1.
4. The polynucleotide of claim 3 which is DNA.
5. The polynucleotide of claim 3 which has the base sequence of SEQ ID NO:2.
6. A recombinant vector comprising the polynucleotide of claim 3.
7. A transformant which has been transformed by the recombinant vector of claim 6.
8. A process for producing the protein of claim 1, or a salt thereof, characterized in that the protein of claim 1 is caused to be produced by culturing the transformant of claim 7.
9. An antibody to the protein of claim 1 or the peptide fragment of claim 2 or a salt thereof.
10. The antibody of claim 9 which is a neutralizing antibody that inactivates signal transduction of the protein of claim 1.
11. A diagnostic agent which is formed comprising the antibody of claim 9.
12. A ligand for the protein of claim 1, or for a salt thereof, which can be obtained by using the protein of claim 1 or the peptide fragment of claim 2 or a salt thereof.
13. A drug which is formed comprising the ligand of claim 12.

14. A method of determining a ligand for the protein of claim 1, or for a salt thereof, which is characterized by using the protein of claim 1 or the peptide fragment of claim 2 or a salt thereof.

15. A method of screening for a compound or a salt thereof which alters the binding of a ligand to the protein of claim 1 or a salt thereof, [said method being] characterized by using the protein of claim 1 or the peptide fragment of claim 2 or a salt thereof.

16. A kit for screening for a compound or a salt thereof which alters the binding of a ligand to the protein of claim 1 or a salt thereof, [said kit being] characterized by comprising the protein of claim 1 or the peptide fragment of claim 2 or a salt thereof.

17. A compound or a salt thereof which alters the binding of a ligand to the protein of claim 1 or a salt thereof, [said compound or salt thereof being] obtainable by using the screening method of claim 15 or the screening kit of claim 16.

18. A drug formed comprising a compound or a salt thereof which alters the binding of a ligand to the protein of claim 1 or a salt thereof, [said compound or salt thereof being] obtainable by using the screening method of claim 15 or the screening kit of claim 16.

19. A polynucleotide which hybridizes to the polynucleotide of claim 3 under highly stringent conditions.

20. A polynucleotide formed comprising a base sequence, and a part of it, that are complementary to the polynucleotide of claim 3.

21. A method of quantifying mRNA for the protein of claim 1 characterized by using the polynucleotide of claim 3 or a part of it.

22. A method of quantifying the protein of claim 1 characterized by using the antibody of claim 9.

23. A method of diagnosing a disease related to a function of the protein of claim 1, [said method being] characterized by using the quantification method of claim 21 or claim 22.

24. A method of screening for a compound or a salt thereof which alters the level of expression of the protein of claim 1, [said method being] characterized by using the quantification method of claim 21.

25. A method of screening for a compound or a salt thereof which alters the amount of protein of claim 1 in the cell membrane, [said method being] characterized by using the quantification method of claim 22.

26. A compound or a salt thereof which alters the level of expression of the protein of claim 1, [said compound or salt being] obtainable by using the screening method of claim 24.

27. A compound or a salt thereof which alters the amount of the protein of claim 1 in a cell membrane, [said compound or salt being] obtainable by using the screening method of claim 25.

Translator's Notes (tn)

[1] "agonist" is misspelled in Japanese.

[2] "agonist" is misspelled in Japanese.

[3] The original uses a term (*korera*) corresponding to "these", even though apparently only one cDNA was isolated. Following "these", I used the plural form for "proteins" for agreement with "these".

[4] Apparent error in original: It would seem that the parenthesis following "etc." should have followed "blood system cell".

[5] This paragraph is identical to the one two paragraphs above in the original too.

[6] The intended meaning is probably that the protein or salt is purified and isolated from the extract, but that is not what the original says.

[7] This sentence does not make sense in Japanese. The intended meaning seems to be " The relationship between the objective nucleic acid and a polynucleotide complementary to at least a portion of the target region and capable of hybridizing with the target is referred to as 'antisense'."

[8] The later part of this sentence (after "known-in-the-art") is confusing in the original, and was rendered as closely as possible in the translation.

[9] " hydrophobic" is misspelled in Japanese.

[10] The intended meaning is probably "part of the polypeptide can be removed".

[11] The hormone system disease examples include items (status peri-afterbirth, cesarean section, etc.) that are not "diseases", but rather seem to be situations in which the invention could be useful. This same wording appears in other locations in the application but is flagged only here.

There were other trivial typographical errors in the original which are not noted here.

Brackets [ ] are used in the translation for two different purposes:

1. They are used to enclose words added by the translator to clarify meaning that is clear in Japanese but could not be conveyed in English without the use of additional words and to make illogical statements logical.
2. They are used where in Japanese double parentheses were used. Brackets in such locations do not contain material added by the translator.